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EDITOR
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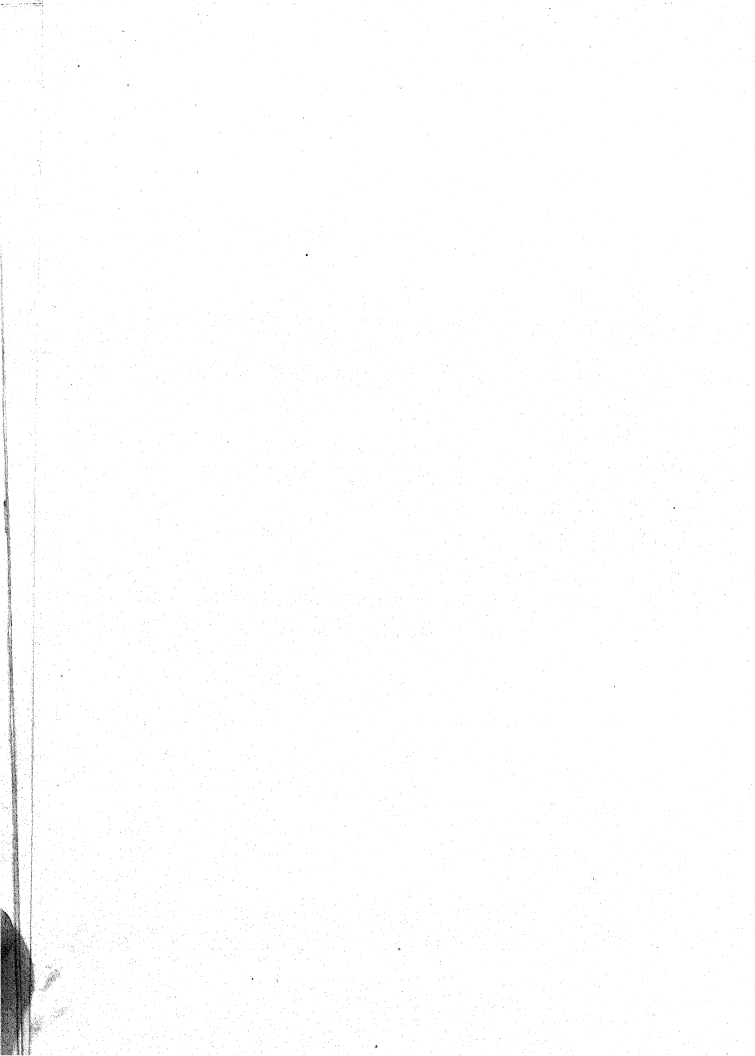
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ERRATA

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P. 204, line 15 from bottom, *omit* (fig. 8d)

P. 205, literature cited, *for* PETERSON *read* PETERSEN, making same correction throughout the paper



THE BOTANICAL GAZETTE

March 1925

ADJUSTMENT OF IRON SUPPLY TO REQUIREMENTS OF SOY BEAN IN SOLUTION CULTURE^{*}

R. P. MARSH AND J. W. SHIVE

(WITH TWO FIGURES)

It has long been understood that green plants deprived of iron lack the ability to form chlorophyll, through the agency of which they are enabled to elaborate carbohydrates from carbon dioxide and water under suitable environmental conditions. Although iron is not a constituent of chlorophyll (24), GRIS (8) more than three-quarters of a century ago showed the necessity of its presence in green plants for the formation of chlorophyll. The significant fact that green plants, grown in culture media without iron, become chlorotic as soon as the reserve iron in the seed is exhausted, is, of course, too well known to need comment.

Quantitative investigation of the frequently observed occurrence of iron chlorosis in plants grown in substrata containing iron has been largely neglected, perhaps on account of its complexity. This somewhat baffling phenomenon, however, has led to considerable speculation as to causes, and, indeed, to some constructive research.

HOFFER and CARR (10) found iron accumulation in the nodes of diseased corn stalks, and they state that with the upper leaves

^{*} Paper no. 182 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

chlorotic these plants contain considerable amounts of immobile iron compounds. MCCALL and HAAG (16), working with wheat, state that some indirect evidence was obtained indicating that plants grown in culture solutions having P_H values ranging from 4.02 to 7.0 sometimes suffer from lack of available iron, or from faulty metabolism resulting from the immobility of the iron within the plants. ARNDT (1), working with corn, found that iron salts when present in injurious concentrations produce a reddish brown discoloration of the lower portions of the nodal areas, owing to an accumulation of an iron combination.

It has been found that great care must be exercised in the use of ferrous salts as sources of iron for plants grown in culture solutions. MAZE (15) found ferrous sulphate, used as a source of iron, toxic to corn. HARTWELL and PEMBER (9) likewise found ferrous sulphate toxic to barley and rye seedlings in Knop's solution, and RUPPRECHT (17) showed ferrous sulphate in a culture solution, when present in concentrations of four parts per million, to be toxic to clover seedlings. JONES and SHIVE (11), employing different increments of iron in the form of ferrous sulphate, found that with some culture solutions it gave excellent results when supplied in relatively very small quantities. These workers showed that in a solution containing ammonium sulphate, the ferrous form of iron is very toxic to plants at exceedingly low concentration, and point out (12) that plants show a marked difference in their response to this form of iron in different types of culture solutions. They further (13) point out that the availability and efficiency of a given iron compound appear to be determined in large measure by the composition of the culture solution, and by the nature of the reaction change induced in the medium by contact with the plant roots. Soluble ferric phosphate has been used with success by DUGGAR (5) for seed plants, and in this connection THATCHER (20) remarks that "only soluble ferric compounds seem to serve as a suitable source of supply of the element, ferrous compounds being usually toxic to plants." Ferric tartrate was found by GILE and CARRERO (6) to be especially available to rice plants, and they have also shown in their work with solution cultures that the amount of iron used has a marked influence upon the availability of this element for rice

plants. Ferric glycono phosphate has been used by MARSH (14) with considerable success in culture media.

TOTTINGHAM and BECK (22) point out that the plant's response to iron during the early stages of growth is dependent upon the amount of iron stored in the seed. CORSON and BAKKE (4) studied both ferrous and ferric phosphates as sources of iron for plants in culture solutions, and found marked differences in their efficiency to supply available iron, and noted also marked differences in the response of different species toward these compounds.

The purpose of the investigations described in the following pages was, in general, to study the relation of the growth of the soy bean to the essential element iron in several types of culture solutions. Studies were made of the conditions under which normal growth of this plant may occur in relation to the iron in the culture medium, and under what conditions chlorosis may occur from lack of iron in the leaves of the plant when an adequate supply is present in the medium. The conditions under which chlorosis may occur in the leaves as the result of iron toxicity were also investigated.

Methods

Great care was taken to expose all plants to similar environmental conditions, in so far as this was possible.

Three types of culture solutions were used. These were the modified Tottingham solution $T_1R_1C_5$ (21), as modified by JONES

TABLE I
GRAM MOLECULAR PARTIAL CONCENTRATIONS OF SALTS IN SOLUTIONS EMPLOYED

Solution	KNO_3	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
Jones and Shive modified Tottingham $T_1R_1C_5$		0.0021	0.0073	0.0071	0.0014
3-salt R_2S_1		0.0053	0.0027	0.0132
Tottingham $T_1R_1C_5$	0.002	0.0021	0.0073	0.0071

and SHIVE (11), who substituted ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations, the 3-salt solution R_2S_1 (18), and the Tottingham solution $T_1R_1C_5$ (21). These solutions were all used at an osmotic concentration of one atmosphere. The formulas of these three solutions are given in table I.

Two-quart colorless glass jars were used for culture receptacles, and the culture solutions were renewed continuously in these by means of "drip and drain" methods, which allowed one liter of new solution to flow into each culture jar at an approximately constant rate during a period of twenty-four hours, while during the same time an equal amount of solution, less the moisture lost by transpiration from the plants, was automatically removed from each culture jar (19). Each culture was continuously aerated by forcing air through the culture solutions.

Three soy bean plants were grown in each culture. The seeds had been germinated in damp sphagnum moss, and when the seedlings were about 5 cm. tall they were carefully selected for uniformity, mounted in two-piece paraffined cork stoppers, and transferred to the culture solutions.

The P_H values of the culture solutions continuously discharged from the culture jars in which the plants were growing were determined at regular intervals during the growth periods of the plants in the different experiments. The colorimetric method was used in making these tests (3, 7), but the readings were occasionally checked by the electrometric method. The readings gave the record of the changes in hydrogen ion concentration of the culture solutions which had been in contact with the plant roots.

The plants of all the cultures of an experiment were harvested on the same day, and the tops and roots were dried separately in an electric oven for three days at 101°C . The dry weights of the tops and roots were then obtained by the chemical balance.

Four iron compounds, Powers-Weightman-Rosengarten's ferric glycerophosphate, soluble ferric phosphate, ferric tartrate, and ferrous sulphate were used in the culture solutions to supply iron in soluble form. A stock solution of each of these iron compounds, of such concentration that each cubic centimeter contained 0.5 mg. of iron, was freshly prepared each day just before being used in the preparation of the culture solutions.

For determining iron in solutions or in the plant tissues, the WOLTER volumetric method (25) was used, and the results obtained by this method were compared with the results obtained from using the WONG colorimetric method (26). Much time was devoted to

the trial and selection of a proper method by which to determine the iron content of the plant tissues and of the culture solutions. The gravimetric methods were eliminated because of the very small amounts of iron considered. In like manner, the potassium permanganate titration method was not employed because of the possibility of oxidation before titration could be completed. It was finally decided to make the analyses by the WOLTER method and to check these results by the WONG method, and in this way acquire confirmatory data. Both of these methods have been successfully used by the biological chemists for the determination quantitatively of exceedingly small amounts of iron in biological fluids. The apparent advantage of these methods over the other quantitative methods is their high sensitiveness, and the fact that the iron is in the stable or ferric form when measured, with no possibility of oxidation or reduction changes taking place.

Iron solubility tests

In order to determine the solubility of the four iron compounds in the several types of culture solutions, the following tests were made. Each of the solutions was adjusted to definite P_H values of 4.6, 5.5, and 6.2. To 250 cc. portions of each of the culture solutions in duplicate were added the required amounts of $N/2$ KOH to bring the hydrogen ion concentration of the solutions to the desired P_H values. To each 250 cc. portion of the different solutions so adjusted was then added 1 mg. of iron in the form of ferric glycono phosphate, soluble ferric phosphate, ferric tartrate, or ferrous sulphate; each form of iron being always supplied at the rate of 4 mg. of iron per liter of culture solution. These iron compounds were added singly in solution form in such concentrations that 2 cc. of the iron solution contained exactly 1 mg. of iron.

These three culture solutions, which had been adjusted to the respective P_H values and supplied with the iron compounds, were allowed to stand in the greenhouse for three days. The solutions were contained in half-liter culture jars carefully covered to protect them from the light. The precipitates, wherever these occurred, were then separated from the culture solution by filtration, and both

the filtrates and the precipitates were analyzed for their iron content by means of the WOLTER volumetric method.

In table II are given the results of the analyses of the solutions. The data represent the average actual amounts in milligrams of iron retained in the filtrates from the 250 cc. portions of the culture solutions after a period of three days, when the iron compounds were present in the solutions in concentrations of 4 mg. of iron per liter of solution.

TABLE II
AMOUNTS OF IRON IN MG. RETAINED IN FILTRATES FROM 250 CC. PORTIONS
OF CULTURE SOLUTIONS

SOLUTION	P _H VALUES	AMOUNT OF IRON IN MG.			
		Ferric glycero phosphate	Soluble ferric phosphate	Ferric tartrate	Ferrous sulphate
Tottingham T ₂ R ₄ C ₅ as modified by Jones and Shive.....	4.6	0.29	0.37	0.47	0.78
	5.5	0.35	0.44	0.55	0.38
	6.2	0.26	0.34	0.48	0.66
3-salt R ₂ Si.....	4.6	0.30	0.43	0.49	0.57
	5.5	0.50	0.47	0.41	0.44
	6.2	0.29	0.17	0.37	0.13
Tottingham T ₂ R ₄ C ₅	4.6	0.20	0.40	0.70	0.70
	5.5	0.35	0.40	0.73	0.84
	6.2	0.20	0.00	0.40	0.00

It is to be emphasized, of course, that the amounts of iron determined in the precipitates, when added to the amounts determined in the filtrates, do not always equal 1 mg., as they should if no iron had been lost in the process of analysis, but in most cases they closely approximate this value.

From the data of table II it is apparent that ferric glycero phosphate has a relatively low solubility in each of the three types of solutions, but its solubility at 4.6 and 6.2 appears to be about the same, both in the Tottingham solution and in the modified Tottingham solution. In each of the three types of solutions its solubility appears to be higher at P_H 5.5 than it is at either a lower or a higher P_H value.

Soluble ferric phosphate shows a relatively high solubility at P_H values of 4.6 and 5.5 in each of the three types of solutions, but is practically insoluble at P_H 6.2, except in the modified Tottingham solution.

Ferric tartrate has a relatively high solubility in each of the three types of culture solutions used, showing the highest solubility, however, in the Tottingham solution. It appears that the reaction of the solution, within the range of P_H values used, has no pronounced influence upon the solubility of this compound in these solutions.

Ferrous sulphate, like soluble ferric phosphate, shows relatively high solubility in each of the three types of solutions at P_H values of 4.6 and 5.5, but is insoluble at P_H values of 6.2, except in the modified Tottingham solution, where it shows relatively high solubility.

Assuming that the availability to the plants of iron in a culture solution is closely correlated with the solubility of the iron compound in the solution, the data here considered suggest in a general way that ferric glycerophosphate could be used to advantage as a source of iron for plants, in culture solutions in which the hydrogen ion concentration is maintained at a high level during contact with the plant roots without much danger of iron toxicity, which is quite likely to occur in plants grown in a culture solution of this type when soluble ferric phosphate or ferrous sulphate is used as a source of iron. It is to be expected that soluble ferric phosphate and ferrous sulphate can have little efficiency as sources of iron for plants in culture solutions with P_H values approaching the neutral point. In solutions of this type, ferric tartrate should prove more effective as a source of iron for plants than any other of the iron compounds here considered.

Experiments

EXPERIMENT I.—This was conducted to obtain, if possible, some experimental evidence as to the conditions under which a high or a low absorption of iron by the plant might take place.

In devising this experiment, it was planned to use different iron compounds in the same culture solution, and also to use the same iron compound in different culture solutions. Eighteen cultures were used and were carried out in duplicate. The cultures were constantly aerated and the culture solution continuously renewed. The experiment was conducted during a period of nine weeks, but before harvesting the plants they were carefully examined for degrees of chlorosis and general appearance with respect to health, vigor, etc. Three series of cultures were included in the experiment, designated as series A, B, and C, according as ferric

glycero phosphate, soluble ferric phosphate, or ferrous sulphate was supplied to the cultures as the source of iron for the plants. The iron compounds in solution form were added to the culture solutions to supply equal amounts of iron per culture. The iron concentrations in milligrams per liter of culture solution for the different intervals during the growth period are given in the footnote of table III.

Iron analyses were made on the total tops of the three plants comprised in each culture. The percentage of iron in the tops was calculated on the dry weight basis. The dry weights of the roots and the yields of iron in the roots, although determined in many cases, were not considered reliable, since it was found that a slight precipitate containing iron had collected on the outside of the roots, which could not readily be removed, in this way producing too high percentages of iron for the roots. There was great variability in the amounts of iron in the roots, and little agreement between the iron contents of the roots of duplicate cultures.

The average P_n values of discharged culture solution during the growth period, the condition of the plants, the dry weights of tops, and the percentage of iron in tops of plants grown in duplicate cultures in series A, B, and C are given in table III. From these data, several interesting facts may be observed. In general, it may be said that high yields of tops correspond to low yields of iron in the plant tops. As a rule, the cultures in which the plants gave no visible indication of toxic effects and were entirely free from chlorosis produced the highest dry weight yields in the respective series.

In the comparison of the plants there were two types of chlorosis easily distinguishable. Plants exhibiting the first type bore pale yellow leaves. This chlorotic condition is characteristic of plants suffering from lack of iron (13), and occurs first in the newly formed leaves. The leaves of plants exhibiting the second type did not present the characteristic yellow appearance from lack of iron, but the older leaves were covered with a yellow and green mottling. Many of the plants which were growing in the modified Totttingham solution exhibited a peculiar brown specking, especially on the unfolding new leaves. This condition was later succeeded by a general mottling of the leaves. All the plants showing the toxic

condition presented an unhealthy appearance, with mottled, dying, or dead leaves (13).

TABLE III

AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHT YIELDS, AND PERCENTAGE OF IRON IN TOPS OF PLANTS OF SERIES A, B, AND C OF EXPERIMENT I*

Culture solution	Average P_H	Condition	Dry weight tops, three plants (gm.)	Percentage iron in tops
A: ferric glycerophosphate				
Modified Tottingham.....	{ 4.60	Normal	8.790	0.025
Average.....	{ 4.50	Normal	10.100	0.019
	4.55		9.445	0.022
3-salt R_2S_1	{ 5.00	Chlorotic	5.880	0.052
Average.....	{ 5.00	Chlorotic	4.420	0.056
	5.00		5.150	0.054
Tottingham $T_1R_1C_5$	{ 5.00	Chlorotic	6.120	0.068
Average.....	{ 5.50	Chlorotic	5.220	0.069
	5.25		5.670	0.068
B: soluble ferric phosphate				
Modified Tottingham.....	{ 4.70	Toxic	5.590	0.073
Average.....	{ 4.80	Toxic	4.540	0.097
	4.75		5.056	0.085
3-salt R_2S_1	{ 5.10	Normal	22.590	0.007
Average.....	{ 5.10	Normal	24.250	0.009
	5.10		23.420	0.008
Tottingham $T_1R_1C_5$	{ 5.20	Normal	11.380	0.023
Average.....	{ 5.20	Normal	15.070	0.022
	5.20		13.225	0.022
C: ferrous sulphate				
Modified Tottingham.....	{ 4.70	Toxic	7.850	0.0500
Average.....	{ 4.40	Toxic	9.520	0.0460
	4.55		8.685	0.0480
3-salt R_2S_1	{ 5.20	Normal	9.420	0.0410
Average.....	{ 5.20	Normal	10.780	0.0420
	5.20		10.100	0.0415
Tottingham $T_1R_1C_5$	{ 4.80	Normal	8.950	0.0390
Average.....	{ 4.90	Normal	8.560	0.0420
	4.85		8.755	0.0405

* Iron additions per liter of culture solution: January 17-29, 0.1 mg.; January 30-31, 0.3 mg.; February 1-12, 0.5 mg.; February 13-19, 1 mg.; February 20-March 16, 2 mg.; total iron added per culture during growth period, 64.9 mg.

In table III this second type of chlorosis is indicated as the toxic condition of the plants. The general tendency shown by the plants of these series, as indicated in this table, was that the plants possessing either the chlorotic or the toxic condition have a higher total percentage of iron than do the normal plants. It may be seen that all the plants showing the toxic condition are to be found in the modified Tottingham solution, the average P_{π} values of which, after being in contact with the plant roots, are indicated in table III as being relatively very low. This condition of the plants when grown in the modified Tottingham solution has been pointed out by JONES and SHIVE (13), who explain the phenomena by suggesting that the presence of ammonium sulphate in a solution, either through its influence on the hydrogen ion concentration of the culture solution in contact with growing plant roots, or the possible effect it may have on cell permeability makes the iron more available, and may account for its toxic effects.

It appears from the data obtained that, in normal growth, activities progress most favorably when the plants contain only a relatively low percentage of iron. The condition in which the iron is present within the plant is also possibly very important. A small amount of mobile iron may become equally distributed in roots, stems, petioles, and leaves, and act efficiently; while a larger amount may either lodge in some portion of the roots, stems, or petioles, or perhaps be distributed in such concentration as to result in retarding development. This, of course, is a question involving many complex phenomena, among which must be considered the relative solubilities of the various iron compounds and the compounds of iron formed within the plant (23). In addition to the condition of the iron compounds, consideration must also be given to the changes taking place in the culture media in which the plants are grown, and the effects produced within the plant by contact with the changing culture media. All these are influenced by the variable environmental complex, which, of course, exert an influence on the efficiency of the iron content.

It may be concluded that relatively very small quantities of iron within the plant promote its normal development. It is observed, however, that the nature of the culture solution determines, in a large measure, the rates of iron absorption, since it has been

shown that the toxic condition occurs in the plants grown in the culture solution containing ammonium sulphate. No chlorosis, however, occurred in these plants as a result of lack of iron; on the other hand, chlorosis resulting from lack of iron occurred in some of the plants grown in the three-salt solution and in the Tottingham solution, and always in these solutions when employed with ferric glycerophosphate as the source of iron.

EXPERIMENT II.—This experiment, like the one preceding, was conducted with the object of determining under what conditions a high or a low absorption of iron may take place. The reason for repeating the experiment was primarily to confirm by further observation the apparently contradictory evidence of the preceding experiment, that plants chlorotic from lack of iron in the leaves, as well as those showing the toxic condition, possessed a higher iron content than do the normally healthy plants.

The method of procedure was similar to that employed in experiment I. The same three types of culture solutions and the same three iron compounds were again employed. The plants were five weeks old when harvested. The numerical data of experiment II are presented in table IV.

Here it is again shown that high yields of tops correspond to low yields of iron in the plants. In general, the dry weight yields of the tops are lower than the dry weight yields shown for the preceding experiment, and the percentages of iron in the tops throughout each series are considerably higher than are those shown for the preceding experiment. The relationships which were observed in the preceding experiment, however, are found to be almost identical with those in the present experiment. The lower yields and the higher percentages of iron shown for the tops in the present experiment may be accounted for by the fact that the data in table III refer to plants nine weeks old, while the data in table IV refer to plants only five weeks old.

As before, there were two types of chlorosis easily distinguishable. The mottled yellow, or toxic type, always occurred in the modified Tottingham solution, and this solution, after being in contact with the roots, possessed a relatively high hydrogen ion concentration during the growth period. No chlorotic plants of the pale yellow type were found in the modified Tottingham solution in

either experiment I or II. The chlorotic plants were to be found distributed in the three-salt and Tottingham solutions.

TABLE IV

AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHT YIELDS, AND PERCENTAGE OF IRON IN TOPS OF PLANTS OF SERIES A, B, AND C OF EXPERIMENT II*

Culture solution	Average P_H	Condition	Dry weight tops, three plants (gm.)	Percentage iron in tops
A: ferric glycono phosphate				
Modified Tottingham.....	{ 4.60 4.50	Normal Normal	6.260 7.200	0.0380 0.0470
Average.....	4.55		6.730	0.0425
3-salt R_2S_1	{ 5.30 5.30	Chlorotic Chlorotic	1.970 2.300	0.0810 0.0800
Average.....	5.30		2.153	0.0805
Tottingham $T_1R_1C_3$	{ 5.30 5.30	Chlorotic Chlorotic	2.870 3.610	0.0700 0.0600
Average.....	5.30		3.240	0.0650
B: soluble ferric phosphate				
Modified Tottingham.....	{ 4.90 4.90	Toxic Toxic	4.000 4.160	0.0730 0.0620
Average.....	4.90		4.800	0.0675
3-salt R_2S_1	{ 5.40 5.40	Chlorotic Chlorotic	5.320 4.900	0.0710 0.0600
Average.....	5.40		5.110	0.0655
Tottingham $T_1R_1C_3$	{ 5.60 5.60	Normal Normal	6.190 5.220	0.0330 0.0550
Average.....	5.60		5.705	0.0440
C: ferrous sulphate				
Modified Tottingham.....	{ 4.60 4.60	Toxic Toxic	4.010 3.140	0.1000 0.1400
Average.....	4.60		3.575	0.1200
3-salt R_2S_1	{ 5.40 5.30	Chlorotic Chlorotic	5.150 4.790	0.0420 0.0330
Average.....	5.35		4.970	0.0375
Tottingham $T_1R_1C_3$	{ 5.50 5.50	Chlorotic Chlorotic	5.450 5.600	0.1500 0.0530
Average.....	5.50		5.525	0.1015

* Iron additions per liter of culture solutions: March 14-21, 0.1 mg.; March 22-26, 0.5 mg.; March 27-31, 1 mg.; April 1-4, 2 mg.; April 5-10, 1 mg.; April 11-16, 2 mg.; total iron added per culture during growth period, 36.3 mg.

As in the preceding experiment, so from the data of this experiment also it appears that small percentages of iron favor the development of a normal healthy condition. The condition in relation to the iron constituents, of course, is influenced by the variable complex of the environment. That relatively small quantities of iron within the plant promote its normal development, and that higher concentrations of iron are not conducive to normal growth may be concluded from the data of the two experiments considered. It is important to note that there is a very narrow range of iron concentration in the culture solutions, under a given set of experimental conditions, which will maintain the plants in a healthy, green condition. A concentration of iron in the medium, either slightly above or below that required to maintain the plants in the normally green condition, will produce either the toxic or the chlorotic conditions noted.

The anomalous condition with respect to the abnormally high iron content in the tops showing both the toxic and the chlorotic condition, which appears repeatedly in the data presented in tables III and IV, will be considered in connection with the data of the following experiment, which was undertaken mainly in an endeavor to account for and to clear up this apparently contradictory evidence.

EXPERIMENT III.—This experiment was conducted to determine, if possible, the distribution of the iron in the tops when the plants had been grown under conditions similar to those of the two preceding experiments.

The modified Tottingham solution, the three-salt solution, and the Tottingham solution were used as before. Ferric glycerol phosphate, soluble ferric phosphate, ferric tartrate, and ferrous sulphate were employed as the sources of iron. The amounts of iron per liter supplied to the solutions during the different intervals are shown in the footnote of table V. The cultures were conducted in duplicate. The plants were grown through a period of thirty-five days, and when harvested the leaves were separated from the stems in each culture and the dry weights of stems and leaves determined separately. After the dry weights were obtained, the leaves and the stems were analyzed separately for their iron content, and the percentages of iron were calculated on the dry weight basis. The numerical data of the experiment are presented in table V.

TABLE V
AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHTS OF LEAVES AND STEMS, AND PERCENTAGE OF IRON IN STEMS AND LEAVES OF PLANTS OF SERIES A, B, C, AND D OF EXPERIMENT III*

LEAVES OF PLANTS OF SERIES 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 1M, 1N, 1O, 1P, 1Q, 1R, 1S, 1T, 1U, 1V, 1W, 1X, 1Y, 1Z, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1JL, 1JM, 1JN, 1JO, 1JP, 1JQ, 1JR, 1JS, 1JT, 1JU, 1JV, 1JW, 1JX, 1JY, 1JZ, 1KA, 1KB, 1KC, 1KD, 1KE, 1KF, 1KG, 1KH, 1KI, 1KJ, 1KK, 1KL, 1KM, 1KN, 1KO, 1KP, 1KQ, 1KR, 1KS, 1KT, 1KU, 1KV, 1KW, 1KX, 1KY, 1KZ, 1LA, 1LB, 1LC, 1LD, 1LE, 1LF, 1LG, 1LH, 1LI, 1LJ, 1LK, 1LL, 1LM, 1LN, 1LO, 1LP, 1LQ, 1LR, 1LS, 1LT, 1LU, 1LV, 1LW, 1LX, 1LY, 1LZ, 1MA, 1MB, 1MC, 1MD, 1ME, 1MF, 1MG, 1MH, 1MI, 1MJ, 1MK, 1ML, 1MM, 1MN, 1MO, 1MP, 1MQ, 1MR, 1MS, 1MT, 1MU, 1MV, 1MW, 1MX, 1MY, 1MZ, 1NA, 1NB, 1NC, 1ND, 1NE, 1NF, 1NG, 1NH, 1NI, 1NJ, 1NK, 1NL, 1NM, 1NO, 1NP, 1NQ, 1NR, 1NS, 1NT, 1NU, 1NV, 1NW, 1NX, 1NY, 1NZ, 1OA, 1OB, 1OC, 1OD, 1OE, 1OF, 1OG, 1OH, 1OI, 1OJ, 1OK, 1OL, 1OM, 1ON, 1OO, 1OP, 1OQ, 1OR, 1OS, 1OT, 1OU, 1OV, 1OW, 1OX, 1OY, 1OZ, 1PA, 1PB, 1PC, 1PD, 1PE, 1PF, 1PG, 1PH, 1PI, 1PJ, 1PK, 1PL, 1PM, 1PN, 1PO, 1PP, 1PQ, 1PR, 1PS, 1PT, 1PU, 1PV, 1PW, 1PX, 1PY, 1PZ, 1QA, 1QB, 1QC, 1QD, 1QE, 1QF, 1QG, 1QH, 1QI, 1QJ, 1QK, 1QL, 1QM, 1QN, 1QO, 1QP, 1QQ, 1QR, 1QS, 1QT, 1QU, 1QV, 1QW, 1QX, 1QY, 1QZ, 1RA, 1RB, 1RC, 1RD, 1RE, 1RF, 1RG, 1RH, 1RI, 1RJ, 1RK, 1RL, 1RM, 1RN, 1RO, 1RP, 1RQ, 1RR, 1RS, 1RT, 1RU, 1RV, 1RW, 1RX, 1RY, 1RZ, 1SA, 1SB, 1SC, 1SD, 1SE, 1SF, 1SG, 1SH, 1SI, 1SJ, 1SK, 1SL, 1SM, 1SN, 1SO, 1SP, 1SQ, 1SR, 1SS, 1ST, 1SU, 1SV, 1SW, 1SX, 1SY, 1SZ, 1TA, 1TB, 1TC, 1TD, 1TE, 1TF, 1TG, 1TH, 1TI, 1TJ, 1TK, 1TL, 1TM, 1TN, 1TO, 1TP, 1TQ, 1TR, 1TS, 1TT, 1TU, 1TV, 1TW, 1TX, 1TY, 1TZ, 1UA, 1UB, 1UC, 1UD, 1UE, 1UF, 1UG, 1UH, 1UI, 1UJ, 1UK, 1UL, 1UM, 1UN, 1UO, 1UP, 1UQ, 1UR, 1US, 1UT, 1UU, 1UV, 1UW, 1UX, 1UY, 1UZ, 1VA, 1VB, 1VC, 1VD, 1VE, 1VF, 1VG, 1VH, 1VI, 1VJ, 1VK, 1VL, 1VM, 1VN, 1VO, 1VP, 1VQ, 1VR, 1VS, 1VT, 1VU, 1VV, 1VW, 1VX, 1VY, 1VZ, 1WA, 1WB, 1WC, 1WD, 1WE, 1WF, 1WG, 1WH, 1WI, 1WJ, 1WK, 1WL, 1WM, 1WN, 1WO, 1WP, 1WQ, 1WR, 1WS, 1WT, 1WU, 1WV, 1WW, 1WX, 1WY, 1WZ, 1XA, 1XB, 1XC, 1XD, 1XE, 1XF, 1XG, 1XH, 1XI, 1XJ, 1XK, 1XL, 1XM, 1XN, 1XO, 1XP, 1XQ, 1XR, 1XS, 1XT, 1XU, 1XV, 1XW, 1XX, 1XY, 1XZ, 1YA, 1YB, 1YC, 1YD, 1YE, 1YF, 1YG, 1YH, 1YI, 1YJ, 1YK, 1YL, 1YM, 1YN, 1YO, 1YP, 1YQ, 1YR, 1YS, 1YT, 1YU, 1YV, 1YW, 1YX, 1YY, 1YZ, 1ZA, 1ZB, 1ZC, 1ZD, 1ZE, 1ZF, 1ZG, 1ZH, 1ZI, 1ZJ, 1ZK, 1ZL, 1ZM, 1ZN, 1ZO, 1ZP, 1ZQ, 1ZR, 1ZS, 1ZT, 1ZU, 1ZV, 1ZW, 1ZX, 1ZY, 1ZZ, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1JL, 1JM, 1JN, 1JO, 1JP, 1JQ, 1JR, 1JS, 1JT, 1JU, 1JV, 1JW, 1JX, 1JY, 1JZ, 1KA, 1KB, 1KC, 1KD, 1KE, 1KF, 1KG, 1KH, 1KI, 1KJ, 1KK, 1KL, 1KM, 1KN, 1KO, 1KP, 1KQ, 1KR, 1KS, 1KT, 1KU, 1KV, 1KW, 1KX, 1KY, 1KZ, 1LA, 1LB, 1LC, 1LD, 1LE, 1LF, 1LG, 1LH, 1LI, 1LJ, 1LK, 1LL, 1LM, 1LN, 1LO, 1LP, 1LQ, 1LR, 1LS, 1LT, 1LU, 1LV, 1LW, 1LX, 1LY, 1LZ, 1MA, 1MB, 1MC, 1MD, 1ME, 1MF, 1MG, 1MH, 1MI, 1MJ, 1MK, 1ML, 1MM, 1MN, 1MO, 1MP, 1MQ, 1MR, 1MS, 1MT, 1MU, 1MV, 1MW, 1MX, 1MY, 1MZ, 1NA, 1NB, 1NC, 1ND, 1NE, 1NF, 1NG, 1NH, 1NI, 1NJ, 1NK, 1NL, 1NM, 1NO, 1NP, 1NQ, 1NR, 1NS, 1NT, 1NU, 1NV, 1NW, 1NX, 1NY, 1NZ, 1OA, 1OB, 1OC, 1OD, 1OE, 1OF, 1OG, 1OH, 1OI, 1OJ, 1OK, 1OL, 1OM, 1ON, 1OO, 1OP, 1OQ, 1OR, 1OS, 1OT, 1OU, 1OV, 1OW, 1OX, 1OY, 1OZ, 1PA, 1PB, 1PC, 1PD, 1PE, 1PF, 1PG, 1PH, 1PI, 1PJ, 1PK, 1PL, 1PM, 1PN, 1PO, 1PP, 1PQ, 1PR, 1PS, 1PT, 1PU, 1PV, 1PW, 1PX, 1PY, 1PZ, 1QA, 1QB, 1QC, 1QD, 1QE, 1QF, 1QG, 1QH, 1QI, 1QJ, 1QK, 1QL, 1QM, 1QN, 1QO, 1QP, 1QQ, 1QR, 1QS, 1QT, 1QU, 1QV, 1QW, 1QX, 1QY, 1QZ, 1RA, 1RB, 1RC, 1RD, 1RE, 1RF, 1RG, 1RH, 1RI, 1RJ, 1RK, 1RL, 1RM, 1RN, 1RO, 1RP, 1RQ, 1RR, 1RS, 1RT, 1RU, 1RV, 1RW, 1RX, 1RY, 1RZ, 1SA, 1SB, 1SC, 1SD, 1SE, 1SF, 1SG, 1SH, 1SI, 1SJ, 1SK, 1SL, 1SM, 1SN, 1SO, 1SP, 1SQ, 1SR, 1SS, 1ST, 1SU, 1SV, 1SW, 1SX, 1SY, 1SZ, 1TA, 1TB, 1TC, 1TD, 1TE, 1TF, 1TG, 1TH, 1TI, 1TJ, 1TK, 1TL, 1TM, 1TN, 1TO, 1TP, 1TQ, 1TR, 1TS, 1TT, 1TU, 1TV, 1TW, 1TX, 1TY, 1TZ, 1UA, 1UB, 1UC, 1UD, 1UE, 1UF, 1UG, 1UH, 1UI, 1UJ, 1UK, 1UL, 1UM, 1UN, 1UO, 1UP, 1UQ, 1UR, 1US, 1UT, 1UU, 1UV, 1UW, 1UX, 1UY, 1UZ, 1VA, 1VB, 1VC, 1VD, 1VE, 1VF, 1VG, 1VH, 1VI, 1VJ, 1VK, 1VL, 1VM, 1VN, 1VO, 1VP, 1VQ, 1VR, 1VS, 1VT, 1VU, 1VV, 1VW, 1VX, 1VY, 1VZ, 1WA, 1WB, 1WC, 1WD, 1WE, 1WF, 1WG, 1WH, 1WI, 1WJ, 1WK, 1WL, 1WM, 1WN, 1WO, 1WP, 1WQ, 1WR, 1WS, 1WT, 1WU, 1WV, 1WW, 1WX, 1WY, 1WZ, 1XA, 1XB, 1XC, 1XD, 1XE, 1XF, 1XG, 1XH, 1XI, 1XJ, 1XK, 1XL, 1XM, 1XN, 1XO, 1XP, 1XQ, 1XR, 1XS, 1XT, 1XU, 1XV, 1XW, 1XX, 1XY, 1XZ, 1YA, 1YB, 1YC, 1YD, 1YE, 1YF, 1YG, 1YH, 1YI, 1YJ, 1YK, 1YL, 1YM, 1YN, 1YO, 1YP, 1YQ, 1YR, 1YS, 1YT, 1YU, 1YV, 1YW, 1YX, 1YY, 1YZ, 1ZA, 1ZB, 1ZC, 1ZD, 1ZE, 1ZF, 1ZG, 1ZH, 1ZI, 1ZJ, 1ZK, 1ZL, 1ZM, 1ZN, 1ZO, 1ZP, 1ZQ, 1ZR, 1ZS, 1ZT, 1ZU, 1ZV, 1ZW, 1ZX, 1ZY, 1ZZ, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1JL, 1JM, 1JN, 1JO, 1JP, 1JQ, 1JR, 1JS, 1JT, 1JU, 1JV, 1JW, 1JX, 1JY, 1JZ, 1KA, 1KB, 1KC, 1KD, 1KE, 1KF, 1KG, 1KH, 1KI, 1KJ, 1KK, 1KL, 1KM, 1KN, 1KO, 1KP, 1KQ, 1KR, 1KS, 1KT, 1KU, 1KV, 1KW, 1KX, 1KY, 1KZ, 1LA, 1LB, 1LC, 1LD, 1LE, 1LF, 1LG, 1LH, 1LI, 1LJ, 1LK, 1LL, 1LM, 1LN, 1LO, 1LP, 1LQ, 1LR, 1LS, 1LT, 1LU, 1LV, 1LW, 1LX, 1LY, 1LZ, 1MA, 1MB, 1MC, 1MD, 1ME, 1MF, 1MG, 1MH, 1MI, 1MJ, 1MK, 1ML, 1MM, 1MN, 1MO, 1MP, 1MQ, 1MR, 1MS, 1MT, 1MU, 1MV, 1MW, 1MX, 1MY, 1MZ, 1NA, 1NB, 1NC, 1ND, 1NE, 1NF, 1NG, 1NH, 1NI, 1NJ, 1NK, 1NL, 1NM, 1NO, 1NP, 1NQ, 1NR, 1NS, 1NT, 1NU, 1NV, 1NW, 1NX, 1NY, 1NZ, 1OA, 1OB, 1OC, 1OD, 1OE, 1OF, 1OG, 1OH, 1OI, 1OJ, 1OK, 1OL, 1OM, 1ON, 1OO, 1OP, 1OQ, 1OR, 1OS, 1OT, 1OU, 1OV, 1OW, 1OX, 1OY, 1OZ, 1PA, 1PB, 1PC, 1PD, 1PE, 1PF, 1PG, 1PH, 1PI, 1PJ, 1PK, 1PL, 1PM, 1PN, 1PO, 1PP, 1PQ, 1PR, 1PS, 1PT, 1PU, 1PV, 1PW, 1PX, 1PY, 1PZ, 1QA, 1QB, 1QC, 1QD, 1QE, 1QF, 1QG, 1QH, 1QI, 1QJ, 1QK, 1QL, 1QM, 1QN, 1QO, 1QP, 1QQ, 1QR, 1QS, 1QT, 1QU, 1QV, 1QW, 1QX, 1QY, 1QZ, 1RA, 1RB, 1RC, 1RD, 1RE, 1RF, 1RG, 1RH, 1RI, 1RJ, 1RK, 1RL, 1RM, 1RN, 1RO, 1RP, 1RQ, 1RR, 1RS, 1RT, 1RU, 1RV, 1RW, 1RX, 1RY, 1RZ, 1SA, 1SB, 1SC, 1SD, 1SE, 1SF, 1SG, 1SH, 1SI, 1SJ, 1SK, 1SL, 1SM, 1SN, 1SO, 1SP, 1SQ, 1SR, 1SS, 1ST, 1SU, 1SV, 1SW, 1SX, 1SY, 1SZ, 1TA, 1TB, 1TC, 1TD, 1TE, 1TF, 1TG, 1TH, 1TI, 1TJ, 1TK, 1TL, 1TM, 1TN, 1TO, 1TP, 1TQ, 1TR, 1TS, 1TT, 1TU, 1TV, 1TW, 1TX, 1TY, 1TZ, 1UA, 1UB, 1UC, 1UD, 1UE, 1UF, 1UG, 1UH, 1UI, 1UJ, 1UK, 1UL, 1UM, 1UN, 1UO, 1UP, 1UQ, 1UR, 1US, 1UT, 1UU, 1UV, 1UW, 1UX, 1UY, 1UZ, 1VA, 1VB, 1VC, 1VD, 1VE, 1VF, 1VG, 1VH, 1VI, 1VJ, 1VK, 1VL, 1VM, 1VN, 1VO, 1VP, 1VQ, 1VR, 1VS, 1VT, 1VU, 1VV, 1VW, 1VX, 1VY, 1VZ, 1WA, 1WB, 1WC, 1WD, 1WE, 1WF, 1WG, 1WH, 1WI, 1WJ, 1WK, 1WL, 1WM, 1WN, 1WO, 1WP, 1WQ, 1WR, 1WS, 1WT, 1WU, 1WV, 1WW, 1WX, 1WY, 1WZ, 1XA, 1XB, 1XC, 1XD, 1XE, 1XF, 1XG, 1XH, 1XI, 1XJ, 1XK, 1XL, 1XM, 1XN, 1XO, 1XP, 1XQ, 1XR, 1XS, 1XT, 1XU, 1XV, 1XW, 1XX, 1XY, 1XZ, 1YA, 1YB, 1YC, 1YD, 1YE, 1YF, 1YG, 1YH, 1YI, 1YJ, 1YK, 1YL, 1YM, 1YN, 1YO, 1YP, 1YQ, 1YR, 1YS, 1YT, 1YU, 1YV, 1YW, 1YX, 1YY, 1YZ, 1ZA, 1ZB, 1ZC, 1ZD, 1ZE, 1ZF, 1ZG, 1ZH, 1ZI, 1ZJ, 1ZK, 1ZL, 1ZM, 1ZN, 1ZO, 1ZP, 1ZQ, 1ZR, 1ZS, 1ZT, 1ZU, 1ZV, 1ZW, 1ZX, 1ZY, 1ZZ, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1JL, 1JM, 1JN, 1JO, 1JP, 1JQ, 1JR, 1JS, 1JT, 1JU, 1JV, 1JW, 1JX, 1JY, 1JZ, 1KA, 1KB, 1KC, 1KD, 1KE, 1KF, 1KG, 1KH, 1KI, 1KJ, 1KK, 1KL, 1KM, 1KN, 1KO, 1KP, 1KQ, 1KR, 1KS, 1KT, 1KU, 1KV, 1KW, 1KX, 1KY, 1KZ, 1LA, 1LB, 1LC, 1LD, 1LE, 1LF, 1LG, 1LH, 1LI, 1LJ, 1LK, 1LL, 1LM, 1LN, 1LO, 1LP, 1LQ, 1LR, 1LS, 1LT, 1LU, 1LV, 1LW, 1LX, 1LY, 1LZ, 1MA, 1MB, 1MC, 1MD, 1ME, 1MF, 1MG, 1MH, 1MI, 1MJ, 1MK, 1ML, 1MM, 1MN, 1MO, 1MP, 1MQ, 1MR, 1MS, 1MT, 1MU, 1MV, 1MW, 1MX, 1MY, 1MZ, 1NA, 1NB, 1NC, 1ND, 1NE, 1NF, 1NG, 1NH, 1NI, 1NJ, 1NK, 1NL, 1NM, 1NO, 1NP, 1NQ, 1NR, 1NS, 1NT, 1NU, 1NV, 1NW, 1NX, 1NY, 1NZ, 1OA, 1OB, 1OC, 1OD, 1OE, 1OF, 1OG, 1OH, 1OI, 1OJ, 1OK, 1OL, 1OM, 1ON, 1OO, 1OP, 1OQ, 1OR, 1OS, 1OT, 1OU, 1OV, 1OW, 1OX, 1OY, 1OZ, 1PA, 1PB, 1PC, 1PD, 1PE, 1PF, 1PG, 1PH, 1PI, 1PJ, 1PK, 1PL, 1PM, 1PN, 1PO, 1PP, 1PQ, 1PR, 1PS, 1PT, 1PU, 1PV, 1PW, 1PX, 1PY, 1PZ, 1QA, 1QB, 1QC, 1QD, 1QE, 1QF, 1QG, 1QH, 1QI, 1QJ, 1QK, 1QL, 1QM, 1QN, 1QO, 1QP, 1QQ, 1QR, 1QS, 1QT, 1QU, 1QV, 1QW, 1QX, 1QY, 1QZ, 1RA, 1RB, 1RC, 1RD, 1RE, 1RF, 1RG, 1RH, 1RI, 1RJ, 1RK, 1RL, 1RM, 1RN, 1RO, 1RP, 1RQ, 1RR, 1RS, 1RT, 1RU, 1RV, 1RW, 1RX, 1RY, 1RZ, 1SA, 1SB, 1SC, 1SD, 1SE, 1SF, 1SG, 1SH, 1SI, 1SJ, 1SK, 1SL, 1SM, 1SN, 1SO, 1SP, 1SQ, 1SR, 1SS, 1ST, 1SU, 1SV, 1SW, 1SX, 1SY, 1SZ, 1TA, 1TB, 1TC, 1TD, 1TE, 1TF, 1TG, 1TH, 1TI, 1TJ, 1TK, 1TL, 1TM, 1TN, 1TO, 1TP, 1TQ, 1TR, 1TS, 1TT, 1TU, 1TV, 1TW, 1TX, 1TY, 1TZ, 1UA, 1UB, 1UC, 1UD, 1UE, 1UF, 1UG, 1UH, 1UI, 1UJ, 1UK, 1UL, 1UM, 1UN, 1UO, 1UP, 1UQ, 1UR, 1US, 1UT, 1UU, 1UV, 1UW, 1UX, 1UY, 1UZ, 1VA, 1VB, 1VC, 1VD, 1VE, 1VF, 1VG, 1VH, 1VI, 1VJ, 1VK, 1VL, 1VM, 1VN, 1VO, 1VP, 1VQ, 1VR, 1VS, 1VT, 1VU, 1VV, 1VW, 1VX, 1VY, 1VZ, 1WA, 1WB, 1WC, 1WD, 1WE, 1WF, 1WG, 1WH, 1WI, 1WJ, 1WK, 1WL, 1WM, 1WN, 1WO, 1WP, 1WQ, 1WR, 1WS, 1WT, 1WU, 1WV, 1WW, 1WX, 1WY, 1WZ, 1XA, 1XB, 1XC, 1XD, 1XE, 1XF, 1XG, 1XH, 1XI, 1XJ, 1XK, 1XL, 1XM, 1XN, 1XO, 1XP, 1XQ, 1XR, 1XS, 1XT, 1XU, 1XV, 1XW, 1XX, 1XY, 1XZ, 1YA, 1YB, 1YC, 1YD, 1YE, 1YF, 1YG, 1YH, 1YI, 1YJ, 1YK, 1YL, 1YM, 1YN, 1YO, 1YP, 1YQ, 1YR, 1YS, 1YT, 1YU, 1YV, 1YW, 1YX, 1YY, 1YZ, 1ZA, 1ZB, 1ZC, 1ZD, 1ZE, 1ZF, 1ZG, 1ZH, 1ZI, 1ZJ, 1ZK, 1ZL, 1ZM, 1ZN, 1ZO, 1ZP, 1ZQ, 1ZR, 1ZS, 1ZT, 1ZU, 1ZV, 1ZW, 1ZX, 1ZY, 1ZZ, 1AA, 1AB, 1AC, 1AD, 1AE, 1AF, 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM, 1AN, 1AO, 1AP, 1AQ, 1AR, 1AS, 1AT, 1AU, 1AV, 1AW, 1AX, 1AY, 1AZ, 1BA, 1BB, 1BC, 1BD, 1BE, 1BF, 1BG, 1BH, 1BI, 1BJ, 1BK, 1BL, 1BM, 1BN, 1BO, 1BP, 1BQ, 1BR, 1BS, 1BT, 1BU, 1BV, 1BW, 1BX, 1BY, 1BZ, 1CA, 1CB, 1CC, 1CD, 1CE, 1CF, 1CG, 1CH, 1CI, 1CJ, 1CK, 1CL, 1CM, 1CN, 1CO, 1CP, 1CQ, 1CR, 1CS, 1CT, 1CU, 1CV, 1CW, 1CX, 1CY, 1CZ, 1DA, 1DB, 1DC, 1DD, 1DE, 1DF, 1DG, 1DH, 1DI, 1DJ, 1DK, 1DL, 1DM, 1DN, 1DO, 1DP, 1DQ, 1DR, 1DS, 1DT, 1DU, 1DV, 1DW, 1DX, 1DY, 1DZ, 1EA, 1EB, 1EC, 1ED, 1EE, 1EF, 1EG, 1EH, 1EI, 1EJ, 1EK, 1EL, 1EM, 1EN, 1EO, 1EP, 1EQ, 1ER, 1ES, 1ET, 1EU, 1EV, 1EW, 1EX, 1EY, 1EZ, 1FA, 1FB, 1FC, 1FD, 1FE, 1FF, 1FG, 1FH, 1FI, 1FJ, 1FK, 1FL, 1FM, 1FN, 1FO, 1FP, 1FQ, 1FR, 1FS, 1FT, 1FU, 1FV, 1FW, 1FX, 1FY, 1FZ, 1GA, 1GB, 1GC, 1GD, 1GE, 1GF, 1GG, 1GH, 1GI, 1GJ, 1GK, 1GL, 1GM, 1GN, 1GO, 1GP, 1GQ, 1GR, 1GS, 1GT, 1GU, 1GV, 1GW, 1GX, 1GY, 1GZ, 1HA, 1HB, 1HC, 1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK, 1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR, 1HS, 1HT, 1HU, 1HV, 1HW, 1HX, 1HY, 1HZ, 1IA, 1IB, 1IC, 1ID, 1IE, 1IF, 1IG, 1IH, 1II, 1IJ, 1IK, 1IL, 1IM, 1IN, 1IO, 1IP, 1IQ, 1IR, 1IS, 1IT, 1IU, 1IV, 1IW, 1IX, 1IY, 1IZ, 1JA, 1JB, 1JC, 1JD, 1JE, 1JF, 1JG, 1JH, 1JI, 1JJ, 1JK, 1J									
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* Iron additions per liter of culture solution: May 23-June 3, 0.2 mg.; June 4-5, 0.5 mg.; June 6-24, 1 mg.; total iron added per culture during growth period, 20.0 mg.

3-salt solution R.S.									
A: Ferric glycerophosphate.....	{5.03	Chlorotic	2.97	1.21	4.18	0.0080	0.0800	0.0260	
Average.....	{5.06	Chlorotic	4.93	1.41	6.34	0.0040	0.0690	0.0180	
	5.04		3.96	1.31	5.26	0.0060	0.0740	0.0220	
B: Soluble ferric phosphate.....	{5.43	Normal	4.81	4.78	9.59	0.0160	0.0190	0.0170	
Average.....	{5.16	Normal	4.39	4.09	8.48	0.0200	0.0150	0.0170	
	5.29		4.60	4.43	9.03	0.0180	0.0170	0.0170	
C: Ferric tartrate.....	{5.36	Slightly chlorotic	3.30	4.36	7.66	0.0170	0.0200	0.0180	
Average.....	{5.36	Slightly chlorotic	3.49	3.01	6.50	0.0100	0.0280	0.0210	
	5.36		3.39	3.68	7.07	0.0165	0.0240	0.0190	
D: Ferrous sulphate.....	{5.43	Normal	5.53	5.10	10.63	0.0130	0.0090	0.0110	
Average.....	{5.26	Normal	5.22	5.32	10.54	0.0130	0.0140	0.0130	
	5.34		5.37	5.21	10.58	0.0130	0.0115	0.0120	
Tottingham solution T.R.C.									
A: Ferric glycerophosphate.....	{5.50	Slightly chlorotic	4.67	4.60	9.27	0.0140	0.0150	0.0140	
Average.....	{5.30	Slightly chlorotic	4.50	5.10	9.60	0.0100	0.0130	0.0110	
	5.40		4.58	4.85	9.43	0.0120	0.0140	0.0125	
B: Soluble ferric phosphate.....	{5.66	Slightly chlorotic	4.87	4.54	9.41	0.0090	0.0210	0.0140	
Average.....	{5.76	Slightly chlorotic	3.96	4.45	8.41	0.0130	0.0320	0.0200	
	5.71		4.41	4.49	8.90	0.0110	0.0270	0.0170	
C: Ferric tartrate.....	{5.73	Slightly chlorotic	4.58	4.88	4.46	0.0090	0.0250	0.0170	
Average.....	{5.83	Slightly chlorotic	4.70	5.18	9.88	0.0080	0.0290	0.0190	
	5.78		4.64	5.03	9.67	0.0085	0.0270	0.0180	
D: Ferrous sulphate.....	{5.80	Slightly chlorotic	4.78	4.84	9.62	0.0130	0.0210	0.0170	
Average.....	{5.80	Slightly chlorotic	4.93	4.69	9.62	0.0170	0.0290	0.0240	
	5.80		4.85	4.76	9.62	0.0150	0.0250	0.0205	

It is here again shown that high yields of tops correspond to low yields of iron, but it is further shown that the iron is fairly uniformly distributed in the stems and leaves of the normally healthy plants. These plants usually produced the highest dry weight yields of tops and showed the lowest iron content for whole tops in the respective series. The plants showing the toxic condition are always to be found in the modified Tottingham solution, and in these the iron content is unevenly distributed, being higher in the stems than in the leaves. It is, however, usually higher in both the stems and leaves than in the corresponding parts of normally healthy plants. The chlorotic plants show a lower concentration of iron in the leaves and a much higher concentration of iron in the stems than do the normally healthy plants. Even the slightly chlorotic plants generally have a higher iron content in the stems than in the leaves. All the chlorotic plants, as well as all the plants showing the toxic condition, have as high or a higher total iron content than do the normal plants. The explanation of the apparent anomaly previously referred to is here made clear. The data of table V show that the abnormally high iron content for entire tops of the chlorotic plants may be accounted for by the fact that exceedingly high concentrations of this element are found in the stem, but relatively very low concentrations in the leaves. It thus appears that very low concentration of iron in the leaves accompanied by abnormally high concentrations of this element in the stems results in the chlorotic conditions observed, since only very small percentages of iron finally reach the leaves, the larger portion of the total iron being lodged in roots, stems, or petioles, and since the small amounts finally reaching the leaves may be by no means uniformly distributed even in these organs. The chlorotic condition resulting from lack of iron in the leaves may thus occur with an abundance of iron in the plant. The plants showing the toxic condition invariably have a high iron concentration throughout, although this is by no means uniformly distributed in stems and leaves.

It is apparent that a small percentage of iron equally distributed throughout the plant promotes its health and vigor. This condition is closely related to the proper adjustment of the available iron

supply in the culture medium. If, however, available iron is present in the culture solutions in relative large amounts, and is absorbed by the plant in excess of that required to maintain a healthy green condition, some of this iron may be precipitated in mass within the roots, stems, or petioles before reaching the leaves, and the leaves may actually become chlorotic because they lack the required concentration of active iron to accompany the process of chlorophyll formation. It appears that this is what actually does occur, since the data of table V show relatively low iron content in the leaves and high iron content in the stems of chlorotic plants.

The solubility of the iron absorbed, of course, may be closely related to the hydrogen ion concentration of the plant juices, a point which has not yet received adequate attention.

These data not only emphasize the conclusions previously reached, but also show that the range in concentration of available iron in the medium, as well as the range of concentration of this element within the plant which will promote vigorous growth and favor the development of healthy green leaves, is very narrow. Concentrations of available iron in the medium, or concentrations of this element within the plant which are very slightly above or below the narrow optimum range tend to produce either the toxic or the chlorotic condition, and these same phenomena are likely to occur when the iron absorbed, even in quantities within the optimum range, is for some reason not fairly uniformly distributed throughout the plant.

EXPERIMENT IV.—The purpose of this experiment was to determine whether the iron supply could be so adjusted that normal plants might be grown in each of the three different solution types with any one of the iron compounds employed as the source of iron for the plants.

Soy bean plants of the Manchu variety were grown as before in constantly aerated and continuously renewed solutions, and were harvested at the end of an experimental period of five weeks. The iron supplies were adjusted, as nearly as could be determined, to meet the requirements of the plants. The appearance of the plants in each culture was used as an index of the amount of iron to be supplied in each case. This iron adjustment was not practiced

in the preceding experiments, but each culture of the experiment was given a definite and like amount.

Table VI presents the numerical data of this experiment. The amounts of iron supplied to each culture solution from day to day are omitted from this table but are given in table VII. Failure,

TABLE VI

AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHT YIELDS, AND PERCENTAGE OF IRON IN TOPS OF PLANTS OF SERIES A, B, C, AND D OF EXPERIMENT IV

Solution	Average P_H	Condition	Dry weight tops, three plants (gm.)	Percentage iron in tops
A: ferric glycerophosphate				
Modified Nottingham $T_1R_1C_5$	4.20	Normal	24.20	0.016
3-salt R_2Sr	6.06	Normal	35.20	0.020
Nottingham $T_1R_1C_5$	6.40	Normal	23.98	0.018
B: soluble ferric phosphate				
Modified Nottingham $T_1R_1C_5$	4.16	Normal	20.15	0.017
3-salt R_2Sr	6.10	Normal	28.47	0.020
Nottingham $T_1R_1C_5$	6.60	Normal	31.07	0.017
C: ferric tartrate				
Modified Nottingham $T_1R_1C_5$	4.13	Normal	30.82	0.020
3-salt R_2Sr	6.10	Normal	26.14	0.014
Nottingham $T_1R_1C_5$	6.60	Normal	26.17	0.018
D: ferrous sulphate				
Modified Nottingham $T_1R_1C_5$	4.16	Normal	17.00	0.017
3-salt R_2Sr	6.00	Normal	27.37	0.012
Nottingham $T_1R_1C_5$	6.63	Normal	35.28	0.016

in some instances, to record the amounts of iron in the form of ferric tartrate and ferrous sulphate added to the modified Nottingham solution, makes the data for these two forms of iron in this solution incomplete, and for this reason they have been omitted from the table.

It is again shown in table VI that the high yields of tops correspond to relatively low iron content. These cultures produced

plants which were free from chlorosis or the toxic condition, and also gave high dry weight yields.

The plants were all very large, dark green, and in every respect gave the appearance of health and vigor. There was a marked degree of uniformity in size and general appearance, regardless of the solution in which they were grown or the compound which furnished the source of iron. It should be noted, however, that the total yield from all the cultures with a single solution type is highest for the three-salt solution, only very slightly inferior for the Tottingham solution, but somewhat lower for the modified Tottingham solution.

The same marked uniformity of the iron content throughout the different series may be observed as indicated for yields, regardless of the marked differences in the hydrogen ion concentration of the discharged solution.

The reason for the marked uniformity in the data here considered and the concomitant healthy vigor of the plants may be explained if table VII is consulted and the daily fluctuations in iron increments are considered. The condition of the plants was constantly observed, and their appearance used as an index of the quantity of iron to be supplied in each case. For example, if the unfolding leaves on any particular day assumed a very faint yellow appearance, the supply of iron was slightly but immediately increased in the culture solution. On the other hand, if the plants on any particular day showed a slight lack of luster in the unfolding leaves, a curled margin of these leaves, or a faint indication of the characteristic mottled appearance in the older leaves, then the iron supply was immediately reduced in quantity, or altogether eliminated for a period. Table VII shows that relatively very small quantities of iron were required in the modified Tottingham solution to maintain the plants in a healthy, vigorous, green condition, but the percentage of iron in the plants grown in this solution is not markedly different from that of those grown in the three-salt or the Tottingham solution. This indicates that the iron supply in this solution containing ammonium sulphate is more available than is that in the other solution types, or that the plants in this solution possess a higher capacity for iron absorption by reason of

TABLE VII
CONCENTRATION OF IRON IN MG. PER LITER OF CULTURE SOLUTION FOR VARIOUS INTERVALS THROUGHOUT GROWTH PERIOD
OF EXPERIMENT IV

INTERVALS	CULTURE SOLUTIONS									
	Modified Tottingham solution FeR_2C_4 (mg.)		3-salt R_2S_3 (mg.)				Tottingham FeR_2C_4 (mg.)			
	F.G.P.*	S.F.P.	F.G.P.	S.F.P.	F.T.	F.S.	F.G.P.	S.F.P.	F.T.	F.S.
June 27-28.....	0.10	0.05	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
29-30.....	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
July 1-2.....	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
3.....	0.20	0.10	0.20	0.10	0.10	0.10	0.20	0.20	0.20	0.1
4.....	0.20	0.10	0.20	0.20	0.20	0.10	0.20	0.20	0.20	0.1
5-6.....	0.30	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.1
7-8.....	0.20	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.1
9-10.....	0.20	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.2
11-12.....	0.40	0.10	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.4
13.....	0.20	0.10	0.40	0.40	0.40	0.60	0.60	0.60	0.60	0.6
14.....	0.20	0.20	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.6
15.....	0.20	0.10	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.6
16.....	0.20	0.00	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.6
17.....	0.20	0.05	0.60	0.80	0.80	0.80	0.80	0.80	0.80	0.8
18.....	0.20	0.10	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.8
19-21.....	0.40	0.10	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.0
22-23.....	0.40	0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.0
24-26.....	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
27.....	0.80	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
28.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
29-30.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
31.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
August 1-3.....	0.40	0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.0
Total.....	9.70	4.05	20.10	19.80	19.20	19.40	20.00	19.80	19.70	19.4

* F.G.P. = ferric glycerate phosphate; S.F.P. = soluble ferric phosphate; F.T. = ferric tartrate; F.S. = ferrous sulphate.

greater permeability of the cell membranes involved in the absorption of this element, as has been suggested by JONES and SHIVE (13). This suggestion is supported by the fact that when the iron concentration in this culture solution was relatively high, the iron content was correspondingly high, as shown in preceding experiments, and these plants frequently showed the unhealthy toxic condition caused by an abnormally high iron content throughout the plant. This condition could not occur in the cultures of the present experiment, because the supply of soluble iron was added to the culture solution only as the plants, by their appearance, seemed to require it. In this way, both iron toxicity and chlorosis were prevented. It is to be suggested, however, that the maintenance of the high hydrogen ion concentration in the modified Tottingham solution (by contact with the roots), as indicated by the P_H values of the discharged solution, may account in a large measure for the high availability of the iron supply in this solution.

It thus appears that when the available iron supply in the medium is properly adjusted to meet the requirements of the plants, any one of the culture solutions here employed, supplied with any one of the compounds used as a source of iron, may be expected to produce healthy, vigorous growth of soy bean plants under cultural conditions similar to those here applied.

Discussion

In order to bring out more clearly the relation between iron content and the conditions of the plants (normal, chlorotic, or toxic) as these have been determined by observation and by methods of comparison, the numerical data of the plants showing these conditions have been brought together from tables III, IV, and V, and are shown diagrammatically in figs. 1 and 2. Fig. 1 represents the data brought together from tables III and IV. The data referring to the percentages of iron in the tops and the total dry weights of all plants showing the normal healthy condition were averaged, and the same was done for all plants showing the toxic and chlorotic condition, and from these average data the diagram of fig. 1 was constructed. It is to be emphasized that the data of the figures are the average values for plants ranging in numbers from a minimum of approximately 36 to a maximum of about 100.

Fig. 1 shows that the average percentage of iron in the tops of all the healthy, vigorous plants possessing normally green leaves was only about one-half as high as it was for plants showing the chlorotic condition characteristic of too little iron in the leaves, and considerably less than one-half as high as that for plants showing the toxic condition resulting from too much iron in the leaves.

The abnormally high average iron content shown for plants having leaves chlorotic from lack of iron, as previously explained, apparently results from the lodgment of iron in the stems and per-

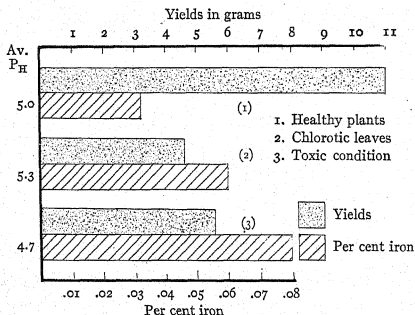


FIG. 1.—Yields and percentage of iron in tops of healthy plants, and in those showing chlorotic or toxic condition.

haps in the roots also, before a supply sufficient to maintain a green healthy color reaches the leaves (fig. 2).

The influence of the chlorotic and toxic conditions of the plants upon growth rates and yields is quite marked (fig. 1). This diagram indicates that the average yield of tops from the cultures producing the chlorotic plants and from those producing plants showing the toxic condition is less than one-half and about one-half, respectively, of the corresponding yield from the cultures which produced healthy, vigorous, normally green plants.

Fig. 2 represents the average data brought together from table V, but the average percentages of iron in stems and leaves are

represented separately. The relations brought out by this diagram, in general, are the same as are those shown by fig. 1. The average percentages of iron for entire tops are much higher for plants showing the chlorotic or the toxic condition than is the corresponding percentage of iron in healthy plants.

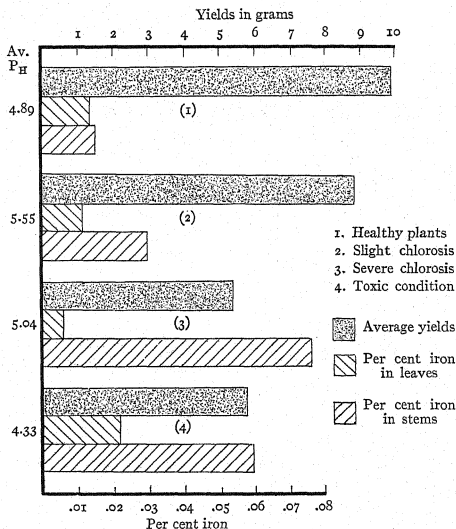


FIG. 2.—Average yields and percentage of iron in leaves and stems of healthy plants, and in leaves and stems of plants showing chlorotic or toxic condition.

It will be observed that the average percentage of iron in healthy plants is relatively quite low in both stems and leaves, and is fairly uniformly distributed. It is to be noted, however, that the average percentage of iron in the leaves of plants showing the marked chlorotic condition is very much lower than is the corresponding percentage of iron in leaves of healthy plants. On the other hand,

the average percentages of iron in the stems of plants showing the chlorotic and the toxic condition are nearly twice as high and five times as high, respectively, as is the corresponding percentage of iron in the stems of healthy plants. This indicates that, although under certain conditions plants may contain an abundant supply of iron, they may still show the characteristic chlorotic conditions of leaves suffering from lack of iron, owing to the fact that the iron is not properly distributed.

At the present time no definite suggestion can be offered with respect to the probable causes of the unequal distribution of iron in these plants, nor why this element should become concentrated in the stems and perhaps in the roots before reaching the leaves. It is possible, however, that this phenomenon may be directly related to the reaction change which the plants themselves may bring about in the medium in which they were grown. For example, it will be observed (fig. 2) that the average P_{π} value (determined at frequent intervals throughout the growth period) of the solutions continuously discharged from the cultures which produced the normally healthy plants varies only slightly from the corresponding initial P_{π} value of these culture solutions. On the other hand, the average P_{π} values of the discharged solution which had been in contact with the roots of plants showing the chlorotic condition, and with those of plants showing the toxic condition, were much higher and somewhat lower, respectively, than were the corresponding initial P_{π} values.

It appears from the foregoing considerations that a small percentage of iron uniformly distributed is requisite for a state of health in the plant. Excessive amounts of iron may either lodge in some portion of the roots, stems, or petioles, or perhaps may be distributed throughout the plant in such concentration as to become toxic. The condition in which the iron is present is undoubtedly also very important. A small amount of mobile iron may become equally distributed in roots, stems, petioles, and leaves, and may act efficiently in the metabolic process or as a catalytic agent in chlorophyll formation.

In making a comparison of the relative efficiency of different culture solutions, it is necessary to take into consideration an

adjustment of the iron supply to the requirements of the plants growing in the culture. Unless the iron supply is properly adjusted, a culture solution will usually not produce good plants.

Summary

1. A study was made of the effects of four soluble iron compounds, used singly, upon the growth and appearance of soy bean plants grown in three types of culture solutions, continuously renewed and constantly aerated.

2. When the iron supply was adjusted from day to day to meet the requirements, large, healthy, vigorous plants were produced, regardless of the type of culture solution or the iron compound employed.

3. When the available iron supply in the medium is slightly in excess of that actually required, the plants may become chlorotic from failure of iron to reach the leaves in the necessary concentration, or they may show the symptoms characteristic of iron toxicity.

4. Quantitative analyses for iron show that healthy green plants possess lower average percentages of iron, on the dry weight basis, than do chlorotic plants or those suffering from iron toxicity. The iron in healthy green plants, however, although relatively low in concentration, appears to be uniformly distributed throughout the stems and leaves, while the iron content of the chlorotic plants is high in the stems and very low in the leaves, and that of the plants suffering from iron toxicity is high throughout.

5. In order to maintain the plants in the healthy green condition, the supply of soluble iron in the culture solution must be maintained at as low a concentration as possible without inducing chlorosis from lack of available iron. A concentration of soluble iron slightly above this optimum may result in iron toxicity from a high content throughout the plant, or chlorosis may occur due to the lodgment of iron in the roots and stems, thus preventing the distribution of the element to the leaves in amounts sufficient to provide adequately for proper chlorophyll formation.

6. Small additions of available iron to the culture solution as the plant appears to require it tend to produce equal distribution throughout, and to keep it in a healthy green condition. The

general appearance of the plant must serve as an index of the iron supply in each case. Definite applications of iron to the culture media at fixed intervals during any given physiological stage of development is not practicable, since the ever changing plant environment has a pronounced influence upon the iron requirement.

7. It appears that a delicate balance exists within the plant which requires that the available iron in the culture medium be limited to a very narrow range of concentrations to produce optimum growth.

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A DISTORTION OF THE 3:1 RATIO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 329

MERLE C. COULTER

Discovery of abnormal ratios

In connection with some experiments conducted in 1919, an ear of corn (710) was produced by self-fertilizing an individual heterozygous for the C factor. As expected, a ratio of 3 colored : 1 white appeared among the grains of this ear. It was further expected that two-thirds of these colored grains would be heterozygous and yield normal 3:1 ratios in the following generation. For the most part, this was the result actually obtained, but three of them gave decided departures from the 3:1 ratio (table I).

TABLE I

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
939.....	11.6	191	25	4.29	29.0	6.76	300,000:1
937.....	11.0	210	26	4.49	33.0	7.35	Over 1,000,000:1
938.....	10.0	207	23	4.43	34.5	7.79	Over 1,000,000:1

Such deviation from the normal ratio was of undoubted significance. Whether this was a genetic significance could be determined by further breeding. The following season, therefore, all of the colored grains of 938 were planted, and a few of 939. As a check, some of the grains of 940 were planted; this was a sib which had shown the normal 3:1 ratio. For the most part, the plants of the next generation were selfed. The results of these selfings appear in tables II, III, and IV.

The progeny of 940 satisfied the expectations, in that exactly one-third of the plants proved to be homozygous, and two-thirds heterozygous for C. The six ratios obtained, however, are perhaps not all as close to the perfect 3:1 as had been expected from previous experience. I believe that the ratio of 140:31 which appears on ear 193 is actually a 3:1 ratio, but the chances are only about one out of 26 that this is the case. Similarly the chances are one out of 13 that

the ratio of ear 195 is a 3:1. The remaining four are normal ratios. In any event, it is clear that the progeny of 940 show nothing like the decided departures from the 3:1 that are in evidence among the progeny of 939 and 938.

The data provided by the progeny of 939 and 938 are apparently quite similar. In the former only a few grains were planted, and

TABLE II
RESULTS OF SELFING PLANTS OF 940 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
198.....	0.0	150	0
192.....	0.0	300	0
191.....	0.0	150	0
193.....	18.1	140	31	3.82	11.75	3.07	25:1
197.....	22.3	73	21	2.83	2.50	Under 1	1:1
190.....	24.3	134	43	3.89	1.25	Under 1	1:1
196.....	24.6	203	66	4.79	1.25	Under 1	1:1
194.....	25.0	111	37	3.57	0.00	Under 1	1:1
195.....	30.3	145	63	4.21	11.00	2.61	12:1

TABLE III
RESULTS OF SELFING PLANTS OF 939 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
154.....	0.00	10	0
152.....	6.8	55	4	2.24	10.75	4.8	825:1
153.....	12.5	42	6	2.02	6.00	3.0	22:1
151.....	12.5	21	3	1.43	3.00	2.1	5:1
150.....	14.3	18	3	1.34	2.25	1.7	3:1
156.....	27.3	32	12	1.94	1.00	Under 1	1:1
155.....	33.3	22	11	1.68	2.75	1.6	3:1

the resulting plants were relatively weak, so that no large ears were obtained. Yet this limited population reveals the same characteristics as does the much larger population of 938, and doubtless the same would have been true of 937.

It is quite evident that the condition which results in deficiency of white grains is heritable. Not that every resulting ear shows such deficiency, for there are clearly some that give all colored and others that give the normal 3:1 ratio; but there are many ears in which an undoubted deficiency of the white grains appears. As for the variation in the amount of white deficiency, that is a secondary problem which will be mentioned later.

TABLE IV

RESULTS OF SELFING PLANTS OF 938 FAMILY (29 EARS GAVE ALL COLORED)

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
119.....	7.5	37	3	1.85	7.00	3.78	92:I
466.....	8.2	201	18	4.32	36.75	8.51	10,000,000:I
433.....	8.6	96	9	2.99	17.25	5.77	10,000:I
429.....	10.3	253	29	4.90	41.50	8.47	10,000,000:I
122.....	10.6	185	22	4.20	29.75	7.08	500,000:I
417.....	10.9	221	27	4.60	35.00	7.61	10,000,000:I
445.....	11.2	230	29	4.70	35.75	7.61	10,000,000:I
427.....	11.7	234	31	4.75	35.25	7.42	2,000,000:I
410.....	12.0	243	33	4.85	36.00	7.42	2,000,000:I
419.....	12.0	257	35	4.99	38.00	7.61	10,000,000:I
412.....	12.2	238	33	4.81	34.75	7.22	1,000,000:I
118.....	12.5	140	20	3.69	20.00	5.42	5,000:I
110.....	12.8	198	29	4.40	27.75	6.31	50,000:I
120.....	13.3	111	17	3.30	15.00	4.55	450:I
431.....	13.9	182	27	4.22	25.25	5.99	20,000:I
425.....	14.5	65	11	2.55	8.00	3.14	28:I
422.....	14.6	169	29	4.11	20.50	4.99	1,300:I
447.....	15.3	198	36	4.47	22.50	5.03	1,400:I
463.....	16.0	199	38	4.50	21.25	4.72	700:I
401.....	16.2	223	43	4.76	23.50	4.94	1,250:I
420.....	16.5	66	13	2.60	6.75	2.60	12:I
461.....	16.7	233	47	4.89	23.00	4.70	600:I
440.....	17.0	83	17	2.92	8.00	2.74	14:I
415.....	17.1	199	41	4.52	19.00	4.20	220:I
462.....	17.9	96	21	3.16	8.25	2.58	11:I
428.....	18.4	200	45	4.57	16.25	3.56	60:I
436.....	18.5	207	47	4.65	16.50	3.53	57:I
116.....	18.5	172	39	4.24	13.75	3.24	33:I
113.....	18.9	77	18	2.85	5.75	2.02	5:I
464.....	19.0	166	39	4.18	12.25	2.93	20:I
467.....	19.9	213	53	4.76	13.50	2.84	17:I
117.....	19.9	97	24	3.21	6.25	1.95	5:I
465.....	21.6	80	22	2.95	3.50	1.19	1.4:I
426.....	22.8	328	97	6.02	9.25	1.53	2:I
446.....	22.8	305	90	5.80	8.75	1.51	2:I
413.....	23.2	195	59	4.65	4.50	Under 1	1:I
121.....	23.3	66	20	2.71	1.50	Under 1	1:I
438.....	24.4	319	103	6.00	2.50	Under 1	1:I
414.....	28.1	151	59	4.23	6.50	1.53	2:I
439.....	33.6	101	51	3.60	13.00	3.89	115:I
460.....	33.9	39	20	2.24	5.25	2.39	8:I

Interpretation

Some years ago an investigator, upon obtaining such results as these, might have been tempted to interpret them as a violation of the Mendelian mechanism. Today, profoundly impressed with the inviolability of this mechanism, one attempts an interpretation of any novel results in Mendelian terms. Several working hypotheses might fit these data. The one which appealed to the writer was as follows.

The race of corn with which I started must have contained a factor L_x , which had never been identified because it had always existed in the homozygous condition.² This factor was linked upon the same chromosome with C, so that the formula of 710 (which was parent of 937-940) was $C-L_x$, $c-l_x$. Self-fertilization of 710 would then have yielded three types of grains: 1 colored, $C-L_x$, $C-l_x$, which would breed true: 2 colored, $C-L_x$, $c-l_x$, which would yield 3:1 on selfing: 1 white, $c-l_x$, $c-l_x$. So long as L_x remained constant, its presence would never be detected. In one of the cells of 710, however, there occurred a mutation which changed the condition $C-L_x$, $c-l_x$ to $C-l_x$, $c-l_x$. This cell evidently existed early enough in ontogeny to be the ancestor of a group of reproductive cells, although whether it was a group of megaspore or microspore mother cells I cannot tell. In any event, this group of reproductive cells produced gametes of the two types, $C-l_x$ and $c-l_x$, the latter type being new. Self-fertilization of 710 caused gametes of this new type to combine with gametes of the two unmodified types, resulting in two new types of zygotes, $C-l_x$, $c-l_x$ and $c-l_x$, $c-l_x$. Three of the colored grains used (937, 938, 939) contained zygotes of the new type $C-l_x$, $c-l_x$; while others (for example, 940) contained zygotes which had been produced by unmutated gametes, and had the formula $C-L_x$, $c-l_x$.

It is in self-fertilization of 937, 938, and 939 that the nature of l_x first becomes apparent. The exact properties of this factor are unknown, but it has the general property of conditioning life, such that any zygote that contains L_x will be normal, while zygotes (and endosperms) lacking L_x cannot develop. Gametes lacking L_x , however, may function (that is, this is a zygotic lethal, not a gametic lethal). If C and L_x were so perfectly linked that no crossing over ever took place between them, plants 937, 938, and 939 would produce, in equal numbers, the two types of gametes, $C-L_x$ and $C-l_x$. The results of self-fertilization would then be, 1- $C-L_x$, $C-L_x$, normal colored: 2 $C-L_x$, $c-l_x$, normal colored: 1 $c-l_x$, $c-l_x$, lethal white. Since this last class would not develop, and since the endo-

² It seems consistent with the notation employed in *Drosophila* to assign the symbol l to the present factor. The subscript distinguishes it from l , which has been assigned to the yellow seedling character in maize. The symbol can be changed, of course, if the present factor proves identical with any previously found, or if the use of such a symbol violates any convention previously established.

sperm ($c-l_1$, $c-l_1$, $c-l_1$) surrounding it could not develop, mature ears would show colored grains only, and the underlying mechanism would not be detected.

Actually, however, C and L_1 are sufficiently separated upon the chromosome so that considerable crossing over takes place between them. Hence there are produced gametes of the following four types:

Non-crossovers, $C-L_1$, and $c-l_1$

Crossovers, $C-l_1$, and $c-L_1$

Both male and female gametes of these four types are produced, since in corn crossing over takes place in both microsporogenesis and megasporogenesis (EMERSON 3). Self-fertilization then results in numerous combinations, the significant features of which are as follows:

(1) There are three combinations from which C is absent, so that the resulting grains will be white if they are able to develop at all.

(a) $c-l_1$, $c-l_1$ (endosperm $c-l_1$, $c-l_1$, $c-l_1$) is lethal; grains will not develop. This results from combination of gametes $c-l_1$ and $c-l_1$ of the non-crossover type. Non-crossover gametes are decidedly more numerous than crossover gametes, hence this combination is relatively frequent.

(b) $c-L_1$, $c-l_1$ (endosperm $c-L_1$, $c-L_1$, $c-l_1$ or $c-L_1$, $c-l_1$, $c-l_1$) is viable; white grains will develop. This results from combination of crossover gamete $c-L_1$ with non-crossover gamete $c-l_1$, and is less frequent than (a), but more frequent than (c).

(c) $c-L_1$, $c-L_1$ (endosperm $c-L_1$, $c-L_1$, $c-L_1$) is viable; white grains will develop. This results from combination of gametes $c-L_1$ and $c-L_1$ of the crossover type, and is relatively rare.

Since (a) is decidedly more frequent than (b) or (c), the majority of potential white grains never develop.

(2) There are several combinations containing C , so that the resulting grains will be colored if they are able to develop at all.

(a) $C-l_1$, $C-l_1$ (endosperm $C-l_1$, $C-l_1$, $C-l_1$) is lethal; grains will not develop. This results from combination of gametes $C-l_1$ and $C-l_1$ of the crossover type and is relatively rare.

(b) $C-l_1$, $c-l_1$ (endosperm $C-l_1$, $C-l_1$, $c-l_1$, or $C-l_1$, $c-l_1$, $c-l_1$) is lethal; grains will not develop. This results from combination of crossover gamete $C-l_1$ with non-crossover gamete $c-l_1$ and is more frequent than (a).

All other combinations containing C will also contain L_r, so that colored grains are developed. The total frequency of these other combinations is so much greater than (a) and (b) that the majority of potential colored grains will develop:

Since the majority of potential white grains never develop and the majority of potential colored grains do develop, the normal Mendelian ratio of 3 colored : 1 white is distorted in the direction of a deficiency of white grains. The amount of this distortion depends upon the relative frequency of crossover and non-crossover gametes. A crossover frequency of about 18 per cent* would yield results such as appeared on ears 937, 938, and 939.

Tests of the working hypothesis

The adequacy of this working hypothesis had to be determined by additional tests. These had to be of such a nature that the results predicted according to this working hypothesis would differ sharply from the results otherwise predicted. Three such tests have been applied.

I. As a matter of convenience, the descriptive title "low-white" may be applied to this mutated race which is characterized by producing a deficiency of white grains when self-fertilized. Will this low-white bring a corresponding deficiency in crosses?

The progeny of 938 and 939 are of various types, some giving all colored when self-fertilized, others giving the normal 3:1, and others giving the characteristic deficiency of whites. It is, of course, only the individuals of this last type that in any event would be expected to show any deficiency of whites in crosses. For purposes of demonstration, therefore, one is obliged to use in the cross the same individual which is self-fertilized; since it is only by self-fertilization that one can discover which individuals are the ones to show the deficiency of whites. This has proved difficult, since the supply of pollen is frequently too small for two successful polli-

* The percentage of crossing over may be computed by means of the following home-made formula, which gives 17.85 per cent in the present case (*n* is total grains, and *w* is white grains):

$$\text{Percentage crossing over} = 100 \cdot \frac{\sqrt{\frac{4n}{3}} - 2\sqrt{\frac{n}{3}} - w}{\sqrt{\frac{4n}{3}}}$$

nations, and it is usually impossible to develop two good ears upon the same plant. As a consequence, although many crosses have been performed with the low-white race, it is in only a few cases that I have been successful in simultaneously selfing and crossing the same individual. These few cases appear in table V. In each case the individual of the low-white race which was used in the cross was known to show a decided deficiency of whites upon self-fertilization.

The cross in question is with "C-tester," a race of which the formula is known to be $c-L_1$, $c-L_1$. The normal result of crossing this with plants heterozygous for C is a ratio of 1 colored:1 colorless. Do the low-whites used in this cross bring a deficiency of whites in the result?

TABLE V
RESULTS OF CROSSING LOW-WHITE WITH C-TESTER

Ear	Low-white as which parent	Percentage white	Colored	Colorless	P.E.	Deviation	Dev./P.E.	Probability
21. 130.....	Male	53.0	195	222	6.89	13.5	1.97	4.4:1
531.....	Female	56.0	51	65	3.63	7.0	1.93	4.2:1
183.....	Female	51.0	103	107	4.89	2.0	Under 1	1:1
24. 40.....	Male	48.7	182	173	6.35	4.5	Under 1	1:1
481.....	Female	48.4	162	152	5.99	5.0	Under 1	1:1
24. 13.....	Male	43.5	39	30	2.80	4.5	1.61	2.6:1
Totals....	732	749	23.42	8.5	Under 1	1:1

It is evident from table V that no deficiency of whites appears in crosses between low-white and C-tester. Nor would one expect any deficiency from the assumption of a zygotic lethal; for the C-tester must always contribute an L_1 , so that the resulting zygote (and endosperm) will be viable.

The exact combinations resulting from this cross are as follows. C-tester produces only one type of gamete, $c-L_1$. Low-white produces four types: non-crossover gametes $C-L_1$ and $c-l_1$ in equal frequency (n), and crossover gametes $C-l_1$ and $c-L_1$ in equal frequency (m , which is less than n). Hence the four possible combinations and their frequencies are: (n) $C-L_1$, $c-L_1$, colored viable: (n) $c-l_1$, $c-L_1$, white viable: (m) $C-l_1$, $c-L_1$, colored viable: (m) $c-L_1$, $c-L_1$, white viable. Then total colored is (n) plus (m), and total white is also (n) plus (m).

Many more crosses were made in which the exact composition of

the low-white parent used was not identified by self-fertilization. The resulting ratios in no case showed a significant deficiency of whites.

II. One might hardly expect to obtain from the low-white race a daughter race which was "high-white," that is, one which was characterized by producing an excess of whites (deficiency of colored grains) when self-fertilized. Yet this very result must be predicted from the assumption of the zygotic lethal.

Among the numerous combinations which result from self-fertilizing the low-white, there is a rare one with the formula $C-l_r, c-L_r$. In this case the previous linkage relationships are reversed, l_r now

TABLE VI

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
547.....	0.0	200	0
549.....	20.2	87	22	3.05	5.25	1.72	3:1
546.....	21.9	50	14	2.34	2.00	Under 1	1:1
551.....	25.0	18	6	1.43	0.00	Under 1	1:1
543.....	25.4	182	62	4.56	1.00	Under 1	1:1
540.....	27.3	8	3	0.97	0.25	Under 1	1:1
545.....	30.9	65	29	2.83	5.50	1.94	4:1
542.....	34.4	82	43	3.27	11.75	3.59	63:1
552.....	34.6	34	18	2.11	5.00	2.37	8:1
541.....	36.4	82	47	3.32	14.75	4.44	370:1
544.....	36.9	41	24	2.35	7.75	3.29	37:1
550.....	39.3	71	46	3.16	16.75	5.30	5,000:1
548.....	40.0	30	20	2.07	7.50	3.62	65:1

being linked in the same chromosome with C , and L_r with c . What result would be expected from self-fertilizing such an individual?

The same forces which brought a deficiency of whites in the previous case must bring a deficiency of colored grains here. Many potential colored grains will be eliminated by combination of the numerous non-crossover gametes $C-l_r$ and $C-L_r$; while only a few potential whites will be eliminated by combination of the less numerous crossover gametes $c-l_r$ and $c-L_r$. Hence the normal 3:1 ratio should be distorted in the direction of a deficiency of colored grains. Calculations based upon the same values used before call for a ratio of about 67 per cent colored:33 per cent white.

In tables III and IV appear a few ratios which are suspiciously close to this predicted high-white condition. Are such individuals truly high-whites, or do they represent merely chance deviations? This question is answered in table VI, the results of self-fertilizing

progeny of the colored grains which occurred on one of these suspected high-whites.

It is evident from table VI that the deficiency of colored grains is heritable. As predicted, a high-white race is produced by the low-white parent race.

TABLE VII
RESULTS OF SELFING PLANTS OF 531 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
24. 110.....	26.6	149	54	4.16	3.25	Under 1	1:1
24. 114.....	26.8	203	77	4.89	7.00	1.43	2:1
24. 115.....	30.6	143	63	4.19	11.50	2.74	14:1
24. 112.....	30.9	183	82	4.75	15.75	3.32	40:1
24. 113.....	34.9	123	66	4.02	18.75	4.64	550:1

TABLE VIII
RESULTS OF SELFING PLANTS OF 183 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
24. 94.....	22.7	341	100	6.13	10.25	1.79	3:1
24. 91.....	23.5	287	88	5.66	5.75	1.02	1.03:1
24. 83.....	23.8	350	109	6.26	5.75	Under 1	1:1
24. 92.....	24.6	362	118	6.40	2.00	Under 1	1:1
24. 80.....	25.3	204	69	4.83	1.75	Under 1	1:1
24. 88.....	25.4	247	84	5.31	1.25	Under 1	1:1
24. 81.....	25.4	288	98	5.74	1.50	Under 1	1:1
24. 82.....	25.5	216	74	4.97	1.50	Under 1	1:1
24. 84.....	25.6	232	80	5.16	2.00	Under 1	1:1
24. 101.....	25.9	281	98	5.69	3.25	Under 1	1:1
24. 100.....	25.9	103	36	3.44	1.25	Under 1	1:1
24. 99.....	26.2	225	80	5.10	3.75	Under 1	1:1
24. 86.....	28.2	102	40	3.48	4.50	1.29	1.6:1
24. 95.....	28.8	227	92	5.22	12.25	2.35	8:1
24. 85.....	30.5	157	69	4.39	12.50	2.85	17:1
24. 96.....	31.0	20	9	1.57	1.75	1.11	1.2:1
24. 89.....	32.7	165	80	4.57	18.75	4.10	200:1
24. 98.....	32.8	180	88	4.78	21.00	4.38	300:1
24. 97.....	34.2	156	81	4.50	21.75	4.83	900:1
24. 87.....	34.5	91	48	3.44	13.25	3.85	100:1
24. 93.....	35.0	130	70	4.13	20.00	4.84	900:1
24. 90.....	36.0	103	58	3.71	17.75	4.79	800:1

III. In the cross between low-white and C-tester, the lethal capacity of the former was concealed, just as any recessive is concealed by its dominant allelomorph. One would expect, therefore, that in the following generation this lethal capacity would become

manifest in at least some of the offspring. Since low-white is the condition that went into the cross, should not low-white be the condition that segregates out again in the second generation? Should not some of the progeny of low-white \times C-tester reveal a deficiency of white grains when self-fertilized?

As a matter of fact, this is not what would be predicted from the assumption of the zygotic lethal. The colored grains produced by crossing low-white with C-tester should be of two types, C- L_x , c- L_x and C- l_x , c- L_x , the former being the more frequent. Self-fertilizing the former should give the normal 3:1 ratio, while self-fertilizing the latter should give a deficiency of *colored* grains, since C- l_x , c- L_x is the formula characteristic of the high-white race. Colored grains of ears 531 and 183 (table V) were planted, and the resulting plants self-fertilized. The results appear in tables VII and VIII.

Again the working hypothesis is confirmed, for it is high-white that regularly segregates out of the cross between low-white and C-tester.

Secondary lines of evidence

In addition to the preceding tests, there are two other lines of evidence that should be mentioned. One lies in the ratio between those colored grains which breed true and those which do not; that is, the ratio between plants homozygous for C and those heterozygous. In the original race, of course, self-fertilization of C- L_x , c- L_x gave 1 C- L_x , C- L_x : 2 C- L_x , c- L_x : 1 c- L_x , c- L_x . Consequently, when colored grains were planted and the resulting plants selfed, one-third of these were expected to give all colored, while the other two-thirds gave some (25 per cent) white grains upon the ears. Previous work has adequately verified this prediction, which is illustrated, upon a small scale, by the progeny of 940 (table II).

The question now arises whether this ratio between pure and heterozygous colored will be different in the low-white and high-white races. Using the first set of data that were available, the ratios which appeared on ears 937-939, one finds a total ratio of 608 colored : 74 white, or 10.85 per cent white, which corresponds to a crossover frequency between C and L_x of approximately 18 per cent. Computations based on this value call for the following relative

frequencies among the different genotypes of the colored class in the low-white race:

Giving all colored when selfed 36.2 per cent

Giving some white when selfed 63.7 per cent

How well these expectations are satisfied by the results obtained (from selfing colored class of 938 and 939) can be judged from table IX.

TABLE IX
RELATIVE FREQUENCY OF PLANTS HOMOZYGOUS AND
HETEROZYGOUS FOR C IN COLORED CLASS OF
LOW-WHITE RACE

	Giving all colored when selfed	Giving some white when selfed
Expected in normal race	25.66	51.33
Observed in low-white	30.00	47.00
Expected in low-white	28.00	49.00

Computations based upon the same crossover value call for the following relative frequencies among the different genotypes of colored grains in the high-white race:

Giving all colored when selfed 16.1 per cent

Giving some white when selfed 83.9 per cent

These expectations are compared with the results obtained in table X.

TABLE X
RELATIVE FREQUENCY OF PLANTS HOMOZYGOUS AND
HETEROZYGOUS FOR C IN COLORED CLASS OF
HIGH-WHITE RACE

	Giving all colored when selfed	Giving some white when selfed
Expected in normal race	4.33	8.66
Observed in low-white	1.00	12.00
Expected in low-white	2.00	11.00

The data in these last tables are meager; yet they at least tend to confirm rather than refute the assumption of a zygotie lethal.

Another secondary line of evidence that might be considered lies in the physical condition of the ears themselves. Those ears

which show either an excess or a deficiency of white grains have presumably had 25 per cent of their potential grains eliminated by the action of the lethal. Hence one might expect such ears to bear approximately three-quarters as many total grains as do the ears with the normal ratio of 3 colored : 1 white, where there is no lethal. Whether this is actually true cannot be determined satisfactorily from examination of the ears produced by self-fertilization of low-white and high-white. When corn has been inbred for several generations, the tendency for "weaklings" to segregate out results in a population of the greatest diversity with respect to the number of grains per ear. The enormous variation due to these causes would

TABLE XI
TOTAL GRAINS PRODUCED ON EARS OF POPULATION
RECORDED IN TABLE VIII

Ear	Percentage white	Total grains	Ear	Percentage white	Total grains
24.94.....	22.7	127.4	24.99.....	26.2	88.3
24.91.....	23.5	108.5	24.86.....	28.2	41.1
24.83.....	23.8	132.9	24.95.....	28.8	92.3
24.92.....	24.6	138.9	24.85.....	30.5	65.4
24.80.....	25.3	79.0	24.89.....	32.7	70.9
24.88.....	25.4	95.8	24.98.....	32.8	77.6
24.81.....	25.4	111.7	24.97.....	34.2	68.6
24.82.....	25.5	83.9	24.87.....	34.5	40.2
24.84.....	25.6	90.3	24.93.....	35.0	57.9
24.101.....	25.9	109.7	24.90.....	36.0	46.6

obscure hopelessly the effect of the lethal upon total number of grains.

There is available, however, another type of population which does not have these shortcomings. The cross between low-white and C-tester brought with it the benefits of heterosis. When the resulting (colored) grains were planted, there was produced a population of relatively vigorous plants with relatively uniform ears. Some of these ears were high-white, due to the activity of the lethal; others lacked the lethal and showed normal 3:1 ratios (table VIII). Here if anywhere the effect of the lethal in reducing the total number of grains should be noticeable. The average number of grains produced on ears which showed the 3:1 ratio was 345.5. In table XI is

recorded the relative number of grains produced on all ears of this population, 100 per cent being equivalent to 345.5 grains.

Clearly there is a tendency for the high-whites to carry less total grains than do the normals. If this is not due to the activity of the lethal, what can be the explanation?

Bearing upon genetics of maize

Two points of interest appear in the bearing of these results upon genetical research in maize. First, as to the nature and identity of the factor $L_1 L_1$. Several lethal factors of the chlorophyll-deficiency type are already well known in maize. In these cases the lethal effect appears in the seedling stage, after the food supply in the seed has been exhausted. For something comparable with the present case, however, one must look to examples of a defect or lethal effect appearing still earlier in ontogeny. There have been reported seeds with no embryo, although the endosperm appears normal (1); also seeds in which both embryo and endosperm, although present, are clearly defective, giving a semi-lethal effect (6). Finally, JONES (5) reports a character of which the extreme expression is absence of both embryo and endosperm, although the pericarp is present. This last situation, which has been shown to be due to a Mendelian recessive, and which, of course, gives a lethal result, certainly represents the same type of behavior as the case in hand. Whether the two cases are identical, may be determined in the future. Some of the ears occurring in these experiments appear to be carrying a few minute "empty pericarps." These are so tiny and irregular, however, that as yet it has not been possible to ascertain any systematic relationship between such aborted seeds and the lethal.

The other point of interest arises from the linkage group into which the present factor falls. Already HUTCHINSON (4) has established the linkage relationships of four endosperm factors, I-C-Sh-Wx. Presumably the present factor will fall into this same group, and should prove useful in enlarging knowledge of linkage phenomena in maize.

Frequency of crossing-over

The tentative crossover frequency of 18 per cent was computed from the ratios which appeared on ears 937, 938, and 939. If this

frequency were constant, the low-whites appearing among the progeny of 938 and 939 should all have possessed close to 10.85 per cent white grains. Instead there appeared an unexpected amount of variation in the relative number of white grains. It was felt that this variation was too wide to be due merely to chance.

Of the various possible explanations of this situation, the one that at present seems to be the most likely is that there is an underlying variation in the frequency of crossing-over. This tentative assumption carries with it the question as to whether environmental or genetic factors are responsible for modifying the crossover frequency; both have been demonstrated in the fruit-fly (2, 8, 9).

With this in mind, selection experiments are now being carried on with the progeny of 938 and 939, and already data have been obtained to justify the following report. Low low-white (6.8 per cent) produces offspring characterized by a lower percentage of white grains than the average (for low-white), and a relatively higher proportion of low-whites as compared with normal 3:1 ratios. Medium (12.5 per cent) and high (18.5 per cent) low-whites produce offspring characterized respectively by a medium and high percentage of white grains, and by a medium and low proportion of low-whites as compared with normal 3:1 ratios. In short, the variation in the relative number of white grains in the low-white race is of genetic significance.

Discussion

The explanation of distorted Mendelian ratios by means of an imaginary zygotic lethal may appear fantastic, yet I believe it is quite consistent with recent discoveries. It is now well established that true mutations are simple factor changes, and that these changes are of a random nature (so far as we can as yet determine). Is it not to be expected that random changes, occurring in the complicated mechanism of the living organism, will more often impair the efficiency than improve it? Certainly this is consistent with the facts, for mutants are more often degenerates than improvements. In fact, the investigation of recent years conveys the impression that lethal mutations are the commonest of all; MULLER and ALTENBURG (7) provide what may be regarded as a demonstration of this point in the fruit fly.

That most mutations are lethal would be more evident if lethal mutations were not so frequently overlooked. Dominant lethal mutations cannot be identified with certainty. Recessive lethal mutations are identified only when (as in the present case) they are linked with some previously known factor that is under observation.

Although dominant and recessive mutations may in reality be equally common, the latter appear to be much more common than the former. This is merely the result of the two facts, that most mutations are lethal, and that recessive lethal mutations can be identified, while dominant lethal mutations cannot. Statistics on observed mutations, therefore, should not be used to demonstrate that there is a greater tendency for recessive than dominant mutations.

The adequacy of mutation as a basis for progressive evolution depends upon whether mutants are ever improvements. Faith in the evolutionary value of mutation has been shaken by claims that no mutant has ever been shown to be an improvement. In answer, two points should be suggested. (1) Would it not be very difficult to recognize as such those rare improvements, or potential improvements, which nature provides through mutation? (2) It is known that dominants may be added by mutation; and this is the only known mode of origin. Many existing dominants are clearly improvements over the corresponding recessives. Have not such dominants been added by mutation in the past?

In objection to this last proposition, the following might be urged. Granting that many existing dominants are such decided improvements over the corresponding recessives that the dominant condition is viable and the recessive lethal, how then may one imagine the circumstances of their origin? For example, can one believe that L_2 (of the present experiments) was suddenly produced by mutation in an organism $l_1 l_1$, when such an organism could not have lived? There can be only one answer, $l_1 l_1$ must have lived. The organism of today is more complicated than the organism of the past; its mode of life involves dependence upon a greater number of mechanical adjustments. Once there existed the organism $l_1 l_1$. Then L_1 appeared and was subsequently utilized in connection with a new type of adaptation. The final perfection of this adaptation

was a step in evolutionary progress; but the price paid was absolute dependence upon L_r . In such a case evolution is irreversible.

In short, if one attempts to visualize evolution in terms of single factor changes alone, the origin of our most important factors by mutation appears logically unsound. The important steps in evolutionary progress doubtless involve several factor changes. One can live on the ground floor without walls or a roof; but if walls and a roof appear, and the family takes up its abode on the roof, the sudden removal of either walls or roof may produce a lethal effect. Our modern organisms are skyscrapers. One should not attempt, therefore, to visualize evolution in terms of single factor changes alone, any more than one should believe that Mendelian factors are the only forces operating to determine expressed hereditary characters.

Summary

1. A race of corn was discovered in which self-fertilization of genotype Cc produces, not 25 per cent white grains, but 10.85 per cent; that is, the normal 3:1 ratio has been distorted in the direction of a deficiency of the recessive class.

2. To account for these results, a working hypothesis is proposed which involves a zygotic lethal factor partially linked with c . If the original genotype $C-L_r, c-L_r$ mutated to $C-L_r, c-l_r$, self-fertilization would eliminate many of the potential whites through the action of the lethal. The exact ratio resulting would depend upon the frequency of crossing-over between c and l_r ; the observed results correspond to about 18 per cent crossing-over.

3. This hypothesis has been confirmed by three breeding tests. (a) Individuals of genotype $C-L_r, c-l_r$, which give a deficiency of whites upon self-fertilization, give no deficiency in crosses with C -tester ($c-l_r, c-L_r$). (b) The low-white race has thrown high-white races (33 per cent white) of composition $C-l_r, c-L_r$. (c) Part of the colored grains from low-white \times C -tester behave as high-white upon self-fertilization.

4. Subsidiary lines of evidence are (a) modified proportion between homozygous and heterozygous colored grains, and (b) deficiency in total number of grains upon those self-fertilized ears which carry the lethal.

5. This factor is very similar to, and possibly identical with, one previously reported by JONES. Presumably it falls into the linkage group I-C-Sh-Wx, which has been worked out by HUTCHINSON.

6. There are indications that frequency of crossing-over may be modified by genetic factors. Data upon this will be presented in a later paper.

7. It is emphasized that lethals are the commonest of mutations. Ideas are presented which may assist in visualizing progressive evolution by factor changes.

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SPATHYEMA FOETIDA

J. MARION SHULL

(WITH PLATES I-IV)

The history of the "skunk cabbage" dates back to its earliest mention by JOHN JOSSELYN, in *New England's rarities discovered*, published in 1672, where he includes it among such plants as are "proper to the country, and have no name." A crudely engraved illustration enables us today to recognize the subject of his description, but if there were any question as to its identity, his statement that "the whole plant sends as strong as a fox" would tend to remove any lingering doubt. Ever since those early days the skunk cabbage has been an object of interest both to botanist and layman; to the latter mainly perhaps because of its pungent, skunklike odor, and because of its seeming extreme precocity in the matter of bloom. In many regions it has come to be looked upon as the earliest of spring flowers. Not having been seen earlier in the season, and then being found in an already spent condition has led to a popular belief that these inflorescences are of extremely rapid, almost spontaneous growth. They are compared with mushrooms for rapidity of development, but this is a mistake.

Impressed by the popular belief in this rapidity of growth, some years ago the writer began a series of observations on a station near Cleveland Park in the District of Columbia, only to find that in this latitude *Spathyema foetida* was hardly to be classed among spring blooming flowers at all. In this locality flowers might have been found in full anthesis at any time between the latter part of November, 1918, and early March of the following spring, a winter of no extreme or long continued cold. Probably few seasons pass at this station without some of the more precocious spathes reaching maturity during the fall or during somewhat protracted periods of warmth in winter. Very likely in more northern regions this fall and winter blooming would occur with less frequency, but it has occasionally been reported from places farther north, and in some cases has led to the mistaken assumption

that it may sometimes flower more than once in the same year. This is not the case, however, since the plant that blooms itself out in November or December does not bloom again the following spring, there being but one annual blooming period for each individual plant, whether it come in fall, winter, or spring. In the region under consideration fall blooming may be looked upon as habitual. It is noteworthy, however, that while these early spathes are developing, all of which are usually destined to be frozen later, others under apparently identical environment as to heat, light, and moisture make no considerable response to the unseasonable warmth, but remain almost entirely quiescent. Thus it would appear that the species is well fortified against such possible disaster as might result from freezing if all the plants pushed their spathes forward with equal promptness under the stimulus of unwonted warmth.

The growth of these earliest appearing spathes, moreover, is not by leaps and bounds, as is the case with so many other spring flowers, but is a gradual and fairly continual process, mostly at relatively low temperatures. Spathes carefully measured and marked with waterproof India ink were observed at intervals from November 6 to December 31, 1918. Later observations were discontinued at this point, owing to accidental damage to a number of the marked plants, but up to this time each successive examination had showed greater or less increase over the next preceding record, the spathes during the whole period having made an average increase of about 4 mm. in diameter and 7 mm. in height, notwithstanding that during much of the time the ground was frozen at the surface. It is probably true that the plant maintains an internal temperature somewhat higher than that of the surrounding air and soil in winter, so that it is not uncommon to find specimens with a small circle about them freed of snow or ice somewhat in advance of its disappearance elsewhere, and under circumstances that apparently cannot be explained except on the assumption of internal warmth. While not being able to find any published data to bear out the statement, nor having had opportunity to corroborate it in any way, it is the writer's understanding that actual measurement of this internal warmth was made some years ago

which showed an internal temperature several degrees higher than that of the surrounding environment. If this is correct it would partially account for the gradual if slow development throughout the greater part of the winter season, as indicated by the measurements here reported.

That the finding of flowers in early autumn led to reports of more than one flowering season per year has already been noted, and can readily be understood, but it is rather difficult to understand how ENGLER (2) should have made the mistake of thinking that *Spathyema* bloomed only in alternate years, foliage one year and flower the next. It is true that to the layman the plant might be rather puzzling, for in the fall or in early spring he might see the spathes with practically no indication of any foliage growth (fig. 12). If in spring, a few weeks later there would be a great mass of light green leafage (fig. 13), which to the casual observer would have no obvious connection with the curious flower found upon the former visit. A few months later he might find an unusual looking object about the size of an average potato, roughened on the outside in a way somewhat suggestive of a pineapple (fig. 15), but so entirely detached from any plant growth then visible as to be beyond his powers of identification, so completely does the foliage disappear. Still later he might find a somewhat scattered handful of something suggestive of ground nuts in appearance, the seeds, now divested of the soft, spongy tissue of the fruit (fig. 14), without even associating them with the peculiar object previously found.

Many features of this interesting plant are not well known, even among botanists. ENGLER pointed out that most of the Araceae are sympodial in their manner of growth, and that *Spathyema* was no exception in this respect, but as he depended on a single dried herbarium specimen for his detailed account of it, it is hardly surprising that he should have gone astray in his description of the succession shoot (1), where he indicates not only too many sheaths or reduced leaves together, but too great a number of ordinary leaves occurring between succeeding spathes. Morphologically, of course, we should make no distinction between these wintering-over sheaths and the more fully expanded leaves of the active vegetative period. Both vary somewhat in number in

different plants, or may grade one into the other to some extent; but so far as the succession shoot is concerned, it invariably consists of two of these units, following which the terminal bud is consumed, one might say, in the development of the inflorescence. Of the two buds in the axils of these two leaves, the upper must always be developed into the new terminal shoot to take the place of the former terminal now converted into a spathe and spadix. The lower of the pair of buds remains of microscopic size until such time as it approaches the outer region of the crown, when it may distinctly be seen without a lens. These may be looked upon as reserve buds and do not ordinarily develop, apparently never do so while the original crown continues to function, and perhaps this is what ROSENDAHL (6) really means when he says, "in no case . . . do they develop into lateral shoots." That these lateral buds do have the power to develop, presumably after many years of dormancy, and do in consequence provide insurance against the destruction of the crown, is well shown in fig. 18, where a portion of an old trunk has freely given rise to lateral growths. Doubtless these lateral growths, had they remained undisturbed, would presently by their very manner of growth have broken themselves free from the parent trunk, and in a few years would have presented all the appearance of individual plants developed from the seed.

In studying the morphology of this plant, FOERSTE (3) explains at some length the deceptiveness of appearances met with when dissecting a mature crown. At first sight it would seem, from their location near the outer circle, that the spathes should have been developed from axillary buds, but on closer examination the ordinary relations of such buds do not hold. There is the added difficulty, of course, that each leaf base makes a practically complete circle, with a series of exactly similar fibrovascular bundles, or shall we say leaf traces, no one of which can be positively determined upon as representing the midvein, so that it is almost impossible to determine the true axis of the leaf. Near the outer portion the bud occurring in the axils of alternate leaves helps to locate the exact axil of the lower leaf of each shoot, but these buds soon become invisible as dissection progresses, and even under a high power lens are frequently missed.

Once it is realized, however, that each spathe in its turn represents the terminal of the whole plant at a certain period, each yielding in its turn to the bud next below with clocklike precision as long as the plant may live, it is easier to interpret what is actually happening. An attempt to diagram this condition is shown in fig. 1, where each circle represents a complete succession shoot with its two leaves, but for clearness the individual leaves are not indicated as extending entirely around the circle. A spathe stands outside the outer circle, the latter representing the axillary bud lying next below the spathe; that is, in the axil of a leaf of the preceding year, which has disappeared. The first or lower leaf is shown with a small white circle at the center, to indicate the presence and location of the lower lateral bud, destined to remain dormant until needed by reason of accident to the upper portion of the plant. The second leaf lies approximately two-fifths around the circle, and in its axil lies the bud represented by the second circle from the outside. This circle lies directly between the second or upper leaf and what is for the moment the axis of the plant here represented by the second spathe. Thus it is clear that the developing and great expansion of this upper of the pair of lateral buds will push the axis or spathe off to a considerable distance, but always directly opposite the upper leaf, the spathe appearing clasped within the margins of the leaf base as seen in figs. 8 and 10. In fig. 1 each succeeding circle, proceeding toward the center, represents the bud lying in the axil of the second leaf of the next preceding circle or shoot, but no attempt has been made to show the large number of such succession shoots that are actually present in any strong crown.

Before dealing further with minute details of dissection, however, it may be well to trace somewhat more connectedly the development of the plant from seed. First, the seed itself is quite unlike most seeds in that there are no seed coats in evidence, these having disappeared during development of the ovary through the absorption of the endosperm and integuments, as reported by ROSENDAHL (5). The mature seeds average about 1 cm. in greatest diameter, and are nearly spherical, except when distorted by pressure due to crowding. Externally they are light brown, while the dense firm

flesh, homogeneous throughout, is of a peculiar bluish white. Opposite the hilum lies the plumule, imbedded in the starchy tuber-like mass of the seed itself, a small slit on the surface adjoining the micropyle serving to show its location. A median longitudinal section of the seed is shown in fig. 6, where the vascular relationship may be noted. The plumule shown under greater magnification (fig. 5) and again in cross-section (fig. 4) is in no respect different from the lateral bud shown in fig. 3, all the same magnification, and quite a number of rudimentary leaves are already clearly in evidence. NUTTALL (4) describes the seed as not appearing to "possess anything like a proper cotyledon," declaring that "in place of a cotyledon there is a sheathing stipule similar to that which is ever after produced; in fact it is viviparous." He is mistaken, of course, in attributing this sheath to stipular origin, whether in the plumule or in the mature plant, since in either case they are merely reduced leaves. ROSENDAHL (5), on the other hand, looks upon the entire fleshy body of the seed as the "cotyledon," in which the plumule is all but completely buried. Shall we say that this seed has already germinated, and so agree with NUTTALL in describing the plant as viviparous? This must depend of course on our definition of germination. In any case this would seem to be the condition in which the seed is prepared to undergo whatever period of inactivity it is capable of sustaining. So far as the writer knows, no determinations have ever been made as to whether or to what extent the seed may undergo drying and still retain its power to develop, or how long it may remain inactive yet viable with a minimum requirement of moisture to prevent shriveling.

The young plant shown still attached to the seed in fig. 2 is thought to be a one-year old plant. At this stage of development, and apparently for a number of years to follow, the growth is monopodial and relatively slow. No one seems to have determined how long the monopodial stage continues, but it is evident that some of these young plants, making often only several leaves in a season, must continue many years before they outgrow this period of infancy. During this time the life of *Spathyema* constitutes one monotonous unfolding of leaf after leaf in exactly the same manner in slow succession, broken only by such periods of rest as the

changing seasons impose, each leaf rolled round and round on itself and completely inclosing the next succeeding leaf, and all rolled in the same direction on the same plant. Only in a single instance among the number of young plants examined was a change in the direction of rolling of the leaf found, and then it manifested itself in serious deformity of the plant. Probably there is considerable variation in the time required to outgrow the monopodial stage, but the crown must have attained a diameter of 20-25 mm. before the change takes place. This is an important period in the life of the young plant, for at this point the younger stage disappears with the converting of the terminal bud for the first time into an inflorescence. After this it will with absolute precision produce two leaves and then an inflorescence, unless through some mishap the growing point should be destroyed, a contingency not often to be reckoned with, since this same point is usually maintained several inches below the surface of the ground and well beyond the reach of animals that sometimes feed upon the leafage; and while damage might be caused by trampling of heavy animals, the mere fact of its swamp habitat would insure against this happening with any frequency.

A young plant having cut off its terminal bud for the first time to form an inflorescence would still be flowerless for a considerable period, possibly for a couple of years; but it will be better at this point to start with a large crown and follow step by step the dissection in detail. The results are shown in condensed form in table I. A large number of crowns of varying sizes were dissected and recorded, and the figures here given are the direct record of a very strong plant with a crown 41 mm. in diameter and as found in November 1918. First there is a large wintering over sheath without leaf expansion, rolled on itself as indicated in the table when looking down on the plant. In the clasp of the leaf base margins there is a wasted spathe. In most plants of this size this spathe would have been sound and there would be no remnants of last year's inflorescence in evidence. In other words, most strong plants at this station produce two mature spathes each season, but there are weaker plants with only one, and yet others that produce three, while an extreme of four has been reported. Next there is

another sheath with slight expansion of leaf at the apex, but the edges overlap in the opposite direction and there is no spathe present. The third sheath, again with slight leaf expansion and

TABLE I
RELATIONSHIPS IN LARGE CROWN OF *Spathyema*, 41 MM. DIAMETER,
DISSECTED IN NOVEMBER

No.	Condition	Direction of roll	Spathe	Season
1.....	Large sheath, no leaf expansion	☉	+aborted	Spring 1919
2.....	" " slight expansion	○	○	
3.....	" " slight expansion	☉	+live 28×92 mm.	
4.....	" " considerable expansion	☉	○	
5.....	Leaf fully formed	☉	+aborted	
6.....	" " "	☉	○	
7.....	" " "	☉	+aborted	
8.....	" " "	☉	○	
9.....	" " "	☉	+aborted	
10.....	" " "	☉	○	
11.....	" " "	☉	+aborted	Spring 1920
12.....	" " "	☉	○	
13.....	" " "	☉	+live 4×10 mm.	
14.....	Rudimentary leaf	☉	○	
15.....	" " "	☉	+live 3×6 mm.	
16.....	" " "	☉	○	
17.....	" " "	☉	+live 2×4 mm.	
18.....	" " "	☉	○	
19.....	" " "	☉	+live	
20.....	" " "	☉	○	
21.....	" " "	+live	Spring 1921
22.....	" " "	○	
23.....	" " "	+live	
24.....	" " "	○	
25.....	" " "	+live	
26.....	" " "	○	
27.....	" " "	+live	
28.....	" " "	○	
29.....	" " "	+live	
30.....	" " "	○	
31.....	" " "	+live	

again reversing the direction of its rolling, clasps a live spathe 28 mm. wide and 92 mm. in height, not yet in anthesis but practically fully grown, and apparently destined to bloom in the spring of 1919. This is followed by a fourth sheath which now shows considerable leaf expansion and is again minus the spathe. Very careful inspection at its axil would probably reveal a minute vegeta-

tive bud, but even at this early point in the dissection they begin to be difficult to find. Fifth in order is found a fully formed leaf, lacking only expansion to make the very large leaf common to the plant as seen in early summer. Beginning at one edge it is tightly rolled many times around itself with much crumpling. Entirely within it lies the next leaf, and within the clasp of the basal margins of the petiole is found a spathe that is no longer alive, as is shown by the discoloration and the membranaceous character of the tissues. The succeeding leaves are much the same, except that they grow smaller and smaller, the sixth, eighth, tenth, and twelfth without accompanying spathes, the seventh, ninth, and eleventh clasping aborted spathes. The thirteenth leaf (fig. 8), however, while still a fully formed leaf like its predecessors, clasps at its base a live spathe 4 mm. wide and 10 mm. high, obviously destined to come into flower one year later than the large spathe associated with the third leaf, or sheath, already mentioned, or in the spring of 1920. Now there is found an abrupt change in character from 13 to 14, the latter (fig. 9) being very definitely rudimentary and destined more than a year in advance to become the wintering-over sheath to protect the bud through that period of cold. Figs. 8 and 9 show both leaves to the same scale. From this point on all the spathes are alive, despite the fact that only 13, 15, and 17, in that immediate region, have the slightest possibility of coming to maturity, and probably not more than two of these will escape. Up to the twentieth leaf dissected off, it has still been possible to determine which way the leaf rolls, and, as shown in the table, there is no break in the rule of constant reversal of direction. The seventeenth leaf with its clasped spathe is shown in fig. 10, and beyond this the height of the leaf is reduced more and more. They become mammiform, occupying a pronounced depression at the center of the crown, whence they were dissected to the final number of 31 (fig. 11), the spathe accompanying this last leaf being shown in fig. 7, and photomicrographs of another at about the same stage in figs. 16 and 17. Since the scheme of annual development is so clearly indicated, just as the number of leaves in the bud of many oaks and other trees is definitely fixed long before growth begins in the spring, it is allowable to set aside a

second series of equal number, and assume that this will represent the following year's quota of leaves. Thus it will appear from table I, that 25 and 27 of this crown, had they been permitted to grow, would have provided the bloom in the spring of 1921, some twenty-seven months after the date of this dissection. The remaining spathes there indicated would fall into the third series of aborted spathes, abortion due to occur two years later.

One is tempted to speculate concerning this suppression of more than fifty per cent of the spathes. This behavior is noted by ROSENDAHL (6), who attempts to explain it on the basis of habit, a habit acquired during an earlier assumed tropical existence, and which it has been unable to leave behind when forced to live under the adverse conditions of alternating winter and summer. In the opinion of the writer this explanation is untenable. In any case such an assumption seems quite unnecessary. The plant is only functioning in a way that is common throughout the plant world. The very manner of its growth, that of converting its terminal bud into an inflorescence at every second turn, leaves no possibility of reducing their number. Consider that the individual fruit is of large size (figs. 14, 15), containing many large seeds consisting of dense albumen. Also, notwithstanding the supposed tropical origin of the species, the plant confines its vegetative growth to a brief two months of the spring, after which the foliage dies off. Obviously it would be impossible for each pair of leaves, large as they are, to provide sufficient photosynthetic action to meet all the needs of the plant otherwise, and also mature a fruit of such size and character. An apple tree, even with the most perfect pollination, drops most of its fruit in order that it may properly mature the remainder. So with *Spathyema*, it is necessary to secure a proper balance between photosynthesis and seed production, which is brought about by the abortion of approximately two-thirds of the spathes inescapably laid down. As to just how this abortion of these particular spathes, attributed by ROSENDAHL (6) to the rigors of winter, is brought about, whether by pressure, by diversion of growth materials, or otherwise, would be a problem for the physiologist. The writer is inclined to think, however, that growth pressure is the means of accomplishing the desirable reduction in number.

To revert to the matter of leaf disposition in the bud and the change that takes place on the transition from monopodial to sympodial manner of growth, it would be interesting to know whether or not the constant alternation of direction is a natural consequence of the latter manner, in a succession shoot of but two leaves. At any rate it is plainly a great advantage in a large, compact, rapidly growing bud, to secure easy and unrestricted expansion, free from all friction of adjacent unrolling leaf surfaces. This may be well illustrated by taking half a dozen strips of paper, rolling the first very closely on itself and the second outside of this but in the opposite direction, and so continuing until all are rolled together. On releasing them note how they all expand at once and throughout the entire roll without the slightest obstruction. This is just what happens with *Spathyema* buds during the extremely rapid vegetative growth of early spring.

Apparently all who have undertaken a detailed account of this species have hesitated over the question of its phyllotaxy, indicating that the leaf arrangement of the mature plants was of a rather high ratio, ROSENDAHL (6) stating it positively as 5-13, but all seem agreed that in the young plant the leaf arrangement is of a lower order, although not risking to say with any assurance just what. ENGLER thought it probably 1-2 in the young plant, changing later to a higher ratio. FOERSTE, while noting the approximate 1-2 appearance of the first pair of leaves, and mentioning the probability of a more complicated arrangement, finally decides that it "seemed reasonable to consider this a case of 1-3 phyllotaxy," crediting such divergence as was more or less apparent to displacements necessarily taking place in so complicated and crowded a structure. There is no denying the difficulty involved in trying to determine the leaf arrangement from flowering crowns with no guiding landmarks during dissection except the recurring more or less developed spathes. Many such dissections were made, inserting pins as each spathe was dissected out, so that the record so obtained could be preserved for comparison with others. These were then compared with idealized diagrams of the various natural arrangements, without obtaining absolute agreement in any case. Various fluctuations occur, probably due to torsion caused either by the

pressure of growth at the crown or by the subsequent great contraction of the tissues as they pass on into the less active portion of the trunk. This same contraction brings the fibrovascular system into such a compact and complicated tangle that any attempt to trace leaf arrangement through that channel is hopeless. We have in fig. 19, however, strong evidence in favor of the common 2-5 arrangement, at least during the monopodial stage. This specimen is apparently the result of a lateral bud coming into activity at considerable depth, and making greatly elongated growth quite unusual for the species in its effort to reach a suitable relationship to the surface. In the old trunk, after its normal contraction, it is impossible even to count leaf scars with any certainty, but here they are far apart, and very distinct until near the crown. A very small bud is to be seen in the lowest axil shown, then at intervals of five scars in each case are three more buds in succession, the compression of the crown making it impossible to follow the series any farther. These four buds, however, are so definitely superimposed in a vertical line on the trunk as to leave no doubt of the arrangement. Even in the crowded crowns, the 2-5 arrangement is approximated, the divergence being irregular, and, on studying cross-sections of lateral buds and embryos (figs. 3, 4), the leaf traces, although in such thick sections as to make actual measurement difficult, are very suggestive of the same arrangement. In view of this evidence, the conclusion is reached that *Spathyema* maintains the 2-5 phyllotaxy through its career from embryo to mature sympodial crowns.

There remains to consider one very interesting speculation concerning this plant. No one seems to know to what age it may attain, but like most slow growing plants it is evidently of very long life. Estimates of as much as seventy-five years have been made, presumably based on the length of trunk present, but in order to understand the uncertainty of any method of estimate we must consider the manner in which the plant counteracts the tendency of all vertical trunks to elongate into the air. This is partly taken care of by the contraction of the trunk itself, as already mentioned, but even so, with an addition of but a millimeter or two each year, and with only terminal growth like a palm, there must eventually be a mounting trunk, with its ultimate risk of being

blown over by wind, or as in the case of trees reaching a maximum height to which the vitalizing forces of the plant may be sent. This possible condition is avoided by *Spathyema* by sending from the crown each season a new circle of strong roots diagonally down to considerable depths, which when well anchored contract their length and drag the whole plant downward with such force that eventually the lower end of an old plant is worn round and smooth like a potato by the thrust into the deeper soil. Thus year by year the crown is maintained at a level somewhat below the surface of the ground, the trunk, with its complement of lateral buds and marked by the scars of leaves that flourished scores of years ago, descends deeper and deeper into the mucky soil. While we may dig it up and determine by its length or by counting as nearly as possible the leaf scars the approximate number of years represented, it is obvious that what has been worn away at the bottom may have been many times greater than that which remains, and it then becomes apparent that *Spathyema* is of indefinite age.

Summary

1. In the District of Columbia *Spathyema foetida* may be found in bloom during moderately warm weather at any time from November to March or April. Each plant has but one blooming period per year, but while some bloom early and usually are frozen later, others under the same conditions of warmth remain unaffected and do not bloom until spring.

2. Growth of spathes is not by leaps and bounds, but is rather slow and continuous, apparently aided by the plant's own internal warmth.

3. Flower, foliage, fruit, and seeds may be seen in such a broken sequence that their relationship may not be very apparent.

4. *Spathyema* pursues a monopodial existence for an indefinite number of years until the first spathe appears, after which it is sympodial with a succession shoot bearing two leaves. The upper axillary bud always becomes the new terminal, while the lower one passes into reserve, developing only in case the terminal crown is destroyed.

5. During the monopodial stage the leaves are all rolled in the

same direction, each entirely outside the next succeeding leaf. On reaching the sympodial stage growth expansion is facilitated by the constant reversal of direction in which the leaves are rolled.

6. Seeds are without seed coats, and the almost buried plumule is indistinguishable from the axillary buds held in reserve.

7. From a large crown as many as 31 leaf units have been dissected. These would provide the full complement of leaves for nearly three years, since it is rare to find a plant with more than 11 leaves expanded in one season. For each second leaf there is a spathe, but more than half of these are ultimately suppressed, probably through the mechanics of growth pressure, with the result of lessening the number of fruits and so securing a proper balance with photosynthesis.

8. Leaf arrangement in this species has been a moot question up to the present time. It is here shown to be definitely 2-5 during the monopodial stage, and this ratio is probably maintained throughout.

9. *Spathyema*, possessing contractile roots that year by year drag it downward into the soil, thereby wearing away by friction the lower end of the trunk, leaves no possible means of even estimating its age, which must be looked upon as indefinite.

CHEVY CHASE, MD.

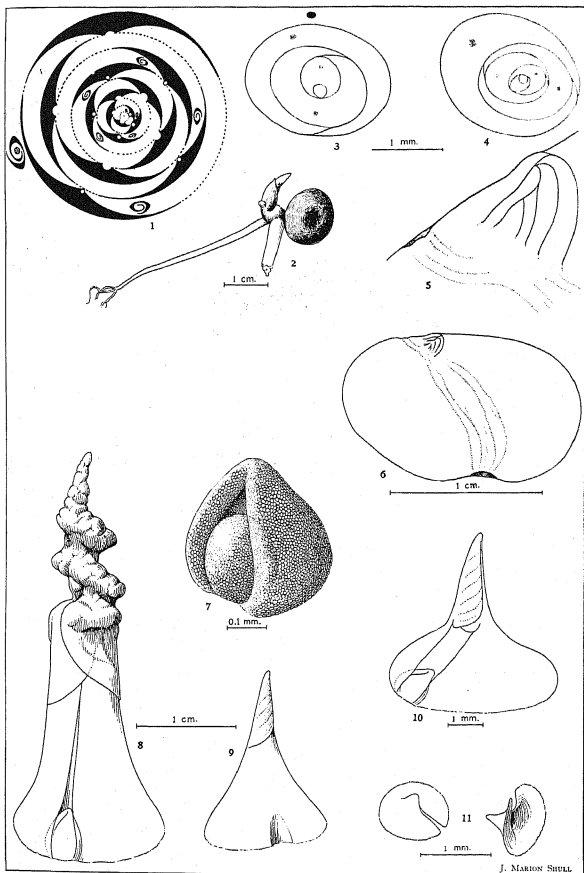
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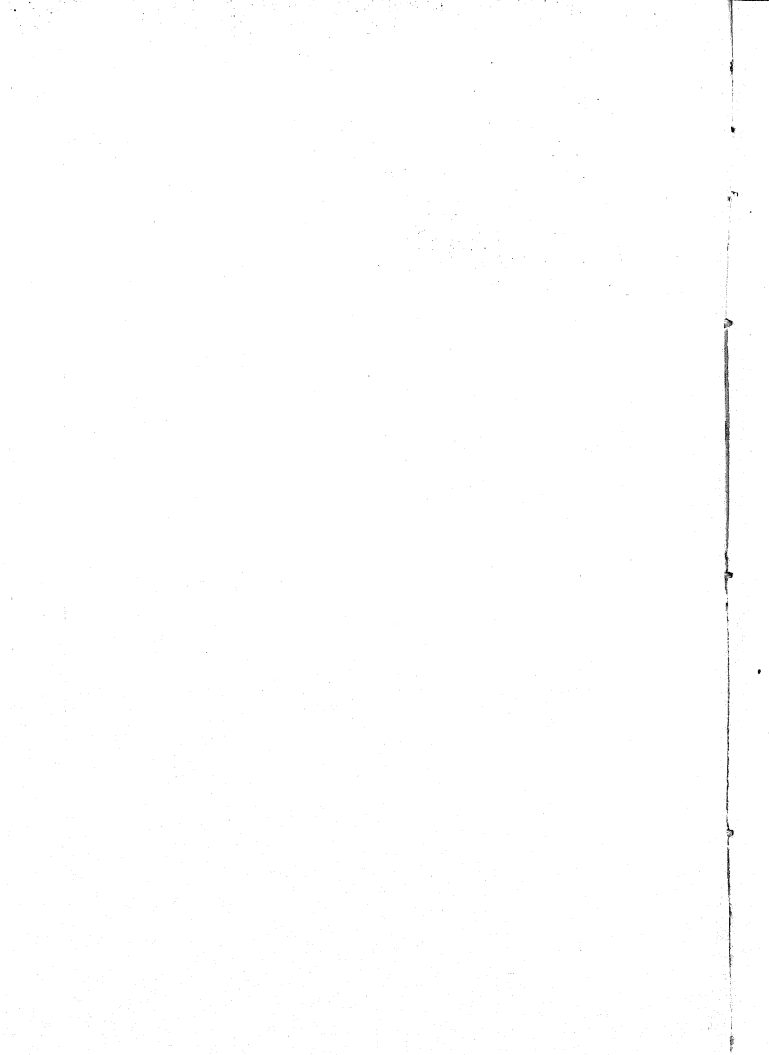
EXPLANATION OF PLATES I-IV

PLATE I

FIG. 1.—Diagram of leaf arrangement and spathe relationship in *Spathyema*; spathe being terminal bud, each successive circle represents

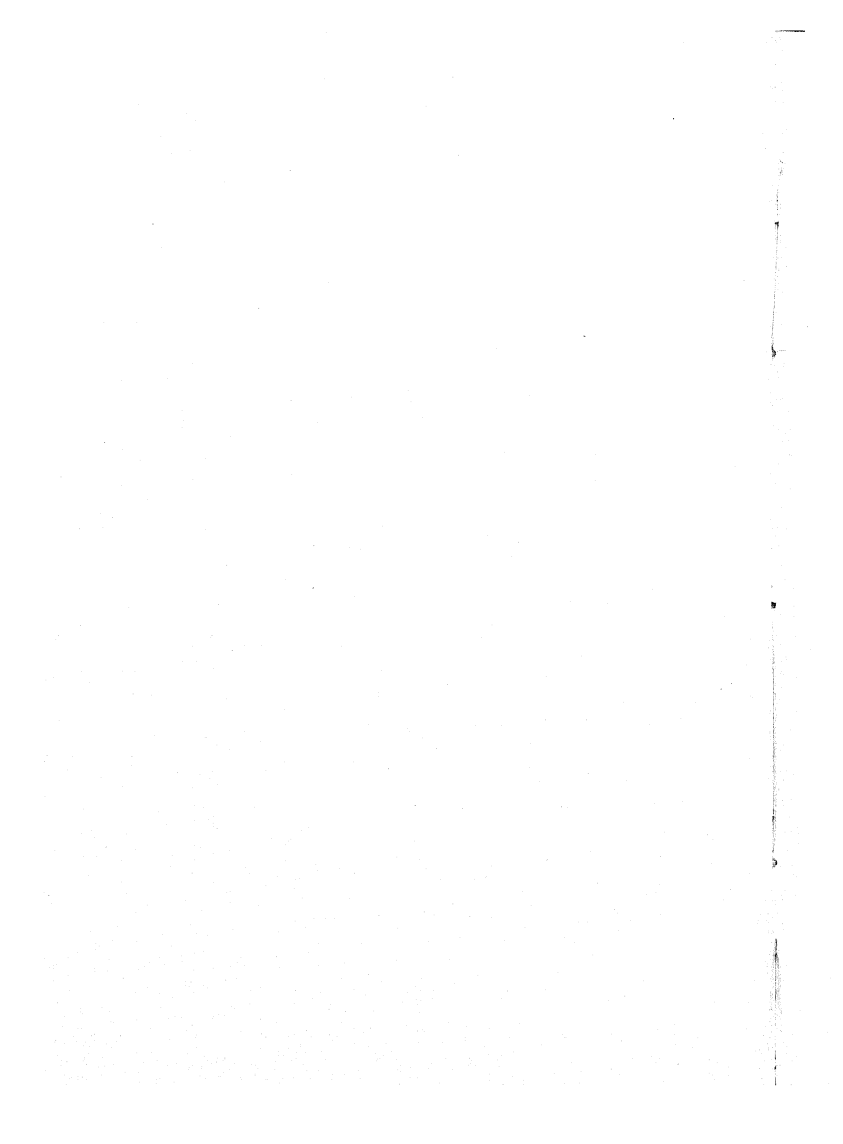


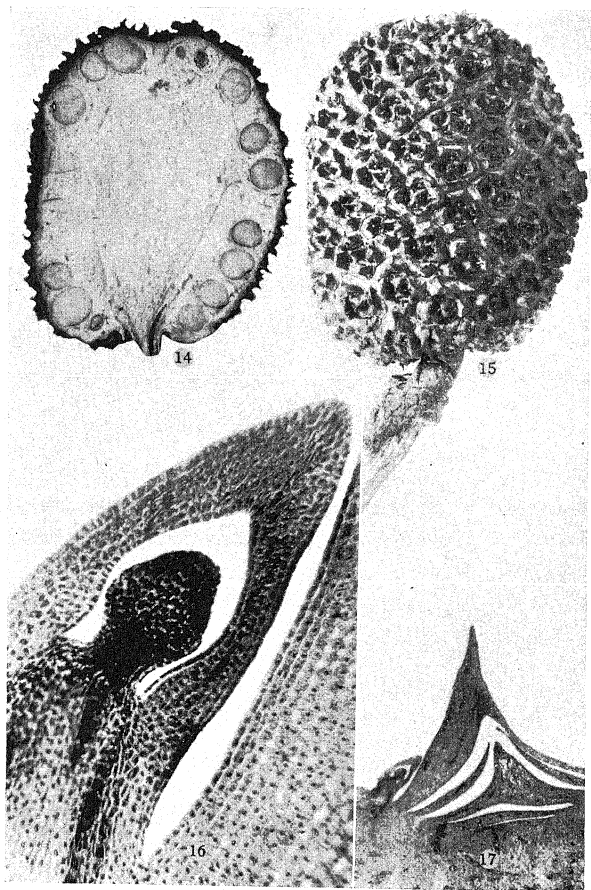
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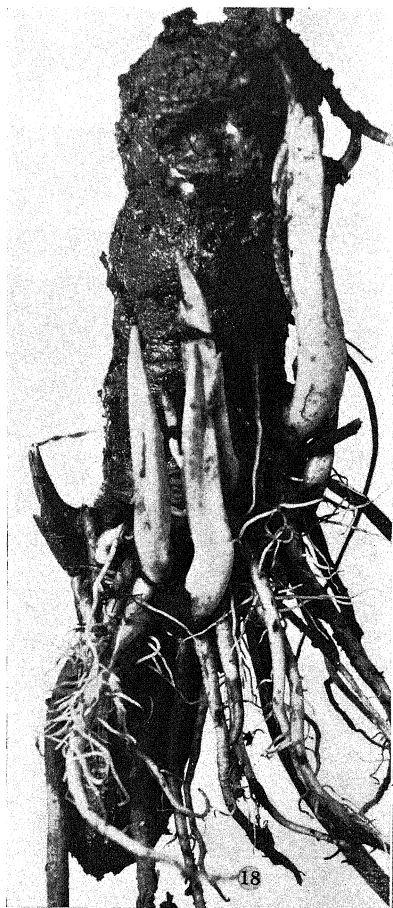
SHULL on SPATHYEMA





SHULL on SPATHYEMA





SHULL on SPATHYEMA

axillary bud lying between this and subtending leaf, missing in case of first spathe shown; each circle may also represent succession shoot of two leaves, lower one subtending a microscopic axillary bud that normally remains dormant, while axillary bud of upper leaf becomes, each in turn, new terminal destined to give rise to two leaves and then become a spathe.

FIG. 2.—One-year-old plant with seed still attached.

FIG. 3.—Axillary bud shown in cross-section.

FIG. 4.—Plumule as found in seed, shown in cross-section.

FIG. 5.—Plumule shown in longitudinal section.

FIG. 6.—Seed in longitudinal section, showing vascular relationship.

FIG. 7.—Last spathe dissected out, apparently not due to bloom until 27 months later (camera lucida).

FIG. 8.—Twelfth leaf unit dissected from vegetative bud in November, last destined to full development during succeeding spring season; probably spathe at base of leaf would have bloomed 15 months later, all those immediately preceding it being aborted.

FIG. 9.—Thirteenth leaf unit is rudimentary leaf which would close season's growth by developing into wintering-over sheath.

FIG. 10.—Seventeenth leaf unit from same bud.

FIG. 11.—Twenty-eighth leaf unit, now reduced to mere mammillary scale (camera lucida).

PLATE II

FIG. 12.—*Spathyema* plant March 6; about $\frac{1}{8}$ natural size.

FIG. 13.—Same plant, taken from same point but with different lens combination, May 21; about $\frac{1}{2}$ natural size.

PLATE III

FIGS. 14, 15.—Fruit in September; slightly reduced.

FIG. 16.—Spathe 27 months in advance of anthesis; $\times 120$.

FIG. 17.—Center of crown with same spathe shown in fig. 16; $\times 14$.

PLATE IV

FIG. 18.—Portion of old trunk freely giving rise to lateral growths from axillary buds held in reserve; natural size.

FIG. 19.—Elongated trunk of young plant, or rather lateral shoot, showing three distinct cycles of leaf scars and axillary buds that indicate 2-5 leaf arrangement; slightly reduced.

CROSSING IRIS PSEUDACORUS AND I. VERSICOLOR

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 330

M. LOUISE SAWYER

(WITH PLATES V-X AND ONE FIGURE)

In the progress of an experiment which was begun in the botanical garden at Grinnell College, Iowa, in the spring of 1918, the writer attempted to produce a hybrid between *Iris Pseudacorus* and *I. versicolor*. Reciprocal crosses were made, and, as has already been reported,¹ the progress was most promising. When the writer left Grinnell for the summer vacation, the ovaries of the cross pollinated flowers were distinctly larger than they were at the time of pollination, were apparently still growing and appeared vigorous, while the ovaries of flowers which had been castrated, covered, and not pollinated were shriveling, had a yellowish color, and were partly separated from their stalk by an abscission layer, if they had not already fallen off.

The promises of the June days were not fulfilled, however, for upon returning to the garden in early September, instead of the harvest of hybrid seeds on *I. Pseudacorus* that was hoped for, only a few withered pods were found still clinging to the plants. In addition to a number of wrinkled seeds, these pods yielded but one plump seed that appeared to be mature, and that one proved to be hollow. A similar attempt the following year had a similar outcome.

These results raised the question as to what occurs following pollination to so stimulate growth and to prolong the life of the ovary and yet fail to produce viable seed. The investigation here reported is concerned with that problem. Both the behavior of the pollen tube and the sequence of the stages in the developing ovules have been examined. The progress of development in ovules of cross pollinated flowers has been compared with that occurring in ovules of flowers which had been self pollinated.

¹ SAWYER, M. LOUISE, Hybridization in *Iris*. Iowa Acad. Sci. 28: 1919.

I. Pseudacorus grows luxuriantly around the botany pond in Hull Court at the University of Chicago, and these plants were the source of most of the material used. Much of the collecting was done in the spring of 1920. The early spring of 1921 resulted in the flowering period ending before the writer could reach the University, and most of the 1922 collection is not yet cut.

In preparing to pollinate, buds were opened just enough to remove the stamens with forceps, and the inflorescences were then covered with bags of thin cloth. Such covering precluded the visits of bees, and the stigma was evidently sufficiently protected by its position. That this was true was indicated by the early death of those flowers which were not hand pollinated after being castrated and protected in this manner. Such flowers drop off in four to seven days after being covered.

The covered flowers which were to be crossed were kept under observation, and during the following day when the stigma was ripe, each flower was pollinated with *I. versicolor* pollen, a tag bearing the data as to the hour and date of pollination was attached, and the flower again covered. The protecting bag was left on until the flower had withered. Collections of the ovaries were made at intervals during the six weeks following pollination. The date of killing, after the early stages, was determined by the appearance of the ovary. It became evident that there was a gradual decrease in the rate of growth of the crossed flowers as compared with that of self pollinated flowers of the same age.

The striking difference in the two sorts of ovaries at the end of six weeks is shown in fig. 1. The ovaries of the crossed flowers were not only smaller, but they remained upright, while the larger and heavier fruits of the self pollinated flowers became pendent. They also differed in form. Three grooves run lengthwise of the crossed ovaries, with the line at the junction of the carpels at the bottom of the furrow, so that transverse sections (fig. 2) have three re-entrant angles separating the locules. The transverse sections of the self pollinated ovaries are nearly terete (fig. 3). The walls of the crossed ovaries are drier and of a more papery texture than those of the selfed ovaries, and their locules in section are lenticular, while in the latter they are nearly circular. The difference in the external ap-

pearance of the ovules is still more significant. In the crossed ovaries the partly developed seeds are shriveled and of varying sizes, the largest not 1/100 of the volume of the selfed seeds. In the crossed

ovaries examined at this age, no ovule showed promise of developing even the hollow seed shell which occasionally occurs.

The collections were killed in chromo-acetic solution to which a little osmic acid was added (about 10 drops per 50 cc. of the killing fluid). They were imbedded in paraffin and cut in serial sections with a rotary microtome. For staining, both Haidenhain's iron-alum haematoxylin and the safranin-gentian violet combination were used.

While both were satisfactory as stains, each had disadvantages. From the 8-nucleate embryo sac stage to the stage of endo-

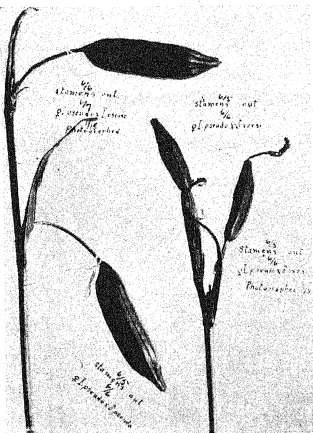


FIG. 1.—Ovaries of *I. pseudacorus* 6 weeks after pollination, showing smaller erect fruit resulting from cross pollinating with *I. versicolor* pollen, and heavier pendent fruit resulting when *I. pseudacorus* pollen is used; photograph by SEDGWICK.

sperm tissue *Iris* ovule sections are difficult to handle. The contents of the embryo sac apparently soften when slides are in aqueous solutions, and during the long washing which is necessary after staining with iron-haematoxylin, they are frequently lost. On the other hand, the surface tensions which develop when slides are transferred from aqueous gentian to high grade alcohols have a similar effect. Even Land's fixative does not prove wholly satisfactory in overcoming these difficulties.

Behavior of pollen tubes

A study of the rate of pollen tube growth was made. Stalks bearing buds of *I. Pseudacorus* were cut, and the stamens removed from

TABLE I

Flower	Pollen applied to stigmas of <i>I. Pseudacorus</i>	Time of pollination	Time of dissection	Time from pollination to dissection		Length of tubes
				Hr.	Min.	
I	{ <i>I. versicolor</i>	9:20	10:20	1		0.05
	{ <i>I. versicolor</i>	9:20	10:22	1		0.10
	{ <i>I. Pseudacorus</i>	9:23	10:26	1		3.00
II	{ <i>I. versicolor</i>	9:25	10:55	1	30	0.06
	{ <i>I. versicolor</i>	9:25	1:25	4		5.00
	{ <i>I. Pseudacorus</i>	9:27	1:30	4		7.00
III	{ <i>I. versicolor</i>	9:30	5:00	7	30	7.00
	{ <i>I. versicolor</i>	9:30	5:35	8	05	20.00
	{ <i>I. Pseudacorus</i>	9:32	5:39	8		12.00
IV	{ <i>I. versicolor</i>	9:35	5:10	7	30	14.00
	{ <i>I. versicolor</i>	9:35	5:35	8		20.00
	{ <i>I. Pseudacorus</i>	9:38	5:40	8		23.00
V	{ <i>I. versicolor</i>	9:40	10:40	1		0.00
	{ <i>I. versicolor</i>	9:40	4:40	7		8.00
	{ <i>I. Pseudacorus</i>	9:42	4:45	7		23.00
VI	{ <i>I. versicolor</i>	9:45	5:47	8		22.00
	{ <i>I. versicolor</i>	9:45	5:50	8	5	24.00
	{ <i>I. Pseudacorus</i>	9:48	5:58	8		24.00
VII	{ <i>I. versicolor</i>	9:50	1:10	3	20	8.00
	{ <i>I. versicolor</i>	9:50	1:15	3	25	5.00
	{ <i>I. Pseudacorus</i>	9:52	1:20	3	28	15.00
VIII	{ <i>I. versicolor</i>	10:00	1:35	3	35	10.00
	{ <i>I. versicolor</i>	10:05	1:37	3	32	8.00
	{ <i>I. Pseudacorus</i>	10:08	1:41	3	33	24.00
IX	{ <i>I. versicolor</i>	10:10	12:05	1	55	0.00
	{ <i>I. versicolor</i>	10:11	3:10	5		0.00
	{ <i>I. Pseudacorus</i>	10:14	12:10	2		0.00
X	{ <i>I. versicolor</i>	10:15	10:45		30	0.00
	{ <i>I. versicolor</i>	10:15	11:15	1		0.06
	{ <i>I. Pseudacorus</i>	10:18	11:48	1	30	0.10

the buds. The following day, as the stigmas ripened, two stigmas in each flower were pollinated with *I. versicolor* pollen, and to the third stigma *I. Pseudacorus* pollen was applied. At intervals the stigmas

were removed and the pollen tubes dissected out and measured. For each dissection the length recorded is that of the longest tubes.

Under the conditions of the experiment it is clearly not possible to have such uniformity of extraneous conditions as is desirable and essential to the securing of significant quantitative results. In this experiment one cannot be sure that all pollen grains from the same flower or even from the same anther have reached the same degree of maturity. Certainly no one can know that "ripe" stigmas of two different flowers, or two stigmas of the same flower, or even two parts of the same stigma are so nearly identical that a given pollen grain would develop tubes at exactly the same rate no matter to which of such stigmas it was applied. In view of these facts and the limited number of dissections which can be reported, the variations in results as indicated in table I are not surprising. Several sets of similar measurements were made, but they gave results so like those of this table that they are not presented.

Attention may be called to one conclusion based on these results. While there is a suggestion that *I. versicolor* pollen tubes tend to grow somewhat less rapidly on *I. Pseudacorus* stigmas than do *I. Psuedacorus* tubes, it is possible for conditions to be such that they grow with practically equal vigor (IV and VI, table I). In view of this fact and the fact that transverse sections of crossed ovaries show pollen tubes in the micropyle (figs. 5, 6), it is evidently not retarded growth of the pollen tubes which is responsible for the failure to secure a hybrid between these two plants.

Ovule

It was the plan to consider four phases in the development of the ovule: (1) rate of growth; (2) behavior of pollen tube; (3) development of endosperm; and (4) development of embryo.

1. RATE OF GROWTH.—An attempt was made to compare the rate of growth of the ovule in selfed and crossed material by measuring the length and breadth of the ovule and embryo sac. The necessity of using only similarly oriented ovules is evident. Only those cut in the median longitudinal plane were of use, which greatly limited the number of ovules available. Furthermore, it became evident, after some 200 measurements had been made, that the

amount of variation in size of ovules of the same age, especially from different ovaries, would make the results meaningless unless a very large number of ovaries of each age could be examined. Lack of sufficient material made it necessary to abandon this phase of the investigation.

2. POLLEN TUBES.—Pollen tubes were first detected in the micropyle 77–78 hours after pollination. The tube does not collapse after penetrating the micropyle, but holds its diameter in a rather remarkable way, both in selfed (fig. 4) and crossed material (fig. 5). It is very common for the tube to branch on reaching the nucellus; usually it merely forks (fig. 6), but occasionally it ends in three branches (fig. 4). HOFMEISTER¹ observed branched pollen tubes in two monocotyledons, *Panikos longifolia* and *Hippeastrum aulicum*. The latter species is one of the Amaryllidaceae, so that this phenomenon has been reported in at least one species which is not far removed from *Iris*.

3. DEVELOPMENT OF ENDOSPERM.—Transverse sections of ovaries killed at the stage when stamens should be removed, if the flower is to be cross pollinated, show the embryo sac not yet fully mature (fig. 7). The polar nuclei have not fused, nor are the antipodal cells organized. Fig. 8 from the antipodal end of an embryo sac, 12 hours after pollination, shows well developed antipodal cells, but the polars are not yet in contact. On the other hand, polars in contact were observed as early as 6 hours after (fig. 9) and as late as 54 hours after pollination (fig. 10). No difference between crossed and selfed ovules could be detected at these stages. The egg apparatus is then intact, and may be surrounded by numerous dark staining metaplasmic bodies, using that term to denote cytoplasmic inclusions. These bodies are probably starch, lying in the cytoplasm (fig. 11).

Each polar nucleus contains a conspicuous nucleolus. The nucleus formed by the fusion of polar nuclei at first shows two nucleoli (fig. 12), presumably one derived from each polar. Later a single large nucleolus is usual and is always the condition when the second male nucleus has arrived (figs. 14, 15). Cases like that shown in fig. 13 suggest that the single nucleolus is the result of fusing. At no

¹ HOFMEISTER, W., Neue Beiträge Zur Kenntniss der Embryobildung der Phanerogamen. Abhandl. Königl. Sachs. Gesell. Wiss. 6: 533–672. 1859.

time was polar fusion delayed as late as the time of the arrival of the male nucleus. This differs from the finding of GUIGNARD,² for he figures *Iris* sp. with the three nuclei in contact.

In crossed material the male nucleus was seen in contact with the nucleus resulting from the fusion of the polars at 77 hours after pollination (fig. 14). The same condition was observed in selfed material killed 78 hours after pollination (fig. 15). In both cases, in the same ovary with ovules showing fusion, there were ovules showing free endosperm nuclei, numbering two to four in crossed and running as high as eight in selfed material. As the selfed ovaries were a little in advance of the crossed ovaries, it is believed that the time difference is not of significance, and that the time of fusion is practically the same. The divisions in the endosperm of both types of material continue. Material taken 11 days after pollination shows the endosperm forming a peripheral layer of numerous nuclei still free (fig. 16). In selfed material the nuclei seem rather more closely packed, and it is believed that they are more numerous. Wall formation has begun by 13 days after pollination in both kinds of material.

As already stated, the egg may show a number of metaplasmic bodies, probably starch, imbedded in its cytoplasm (fig. 11). In self pollinated material such bodies appear abundantly in the embryo sac cytoplasm as well, at the time of fertilization (fig. 22). These were not seen in the crossed material, and this fact is the earliest indication of a difference in the nutritive content of the two endosperms. As time goes on the difference becomes pronounced. Selfed ovules develop an endosperm, which continues to carry a load of these metaplasmic bodies (fig. 20). Crossed material after 20 days shows in the occasional ovule which is still plump an endosperm tissue, not filling the cavity, and made up of thin walled cells with tenuous cytoplasm carrying few metaplasmic bodies, although their nuclei appear normal except for an unusual number of nucleoli (fig. 17). At 26 days the cavity of the ovule was filled with tissue, but it was like well advanced pith, the cells thin walled and almost without contents (fig. 18). In the more vigorous of the ovules, in those ovaries which were still living at 6 weeks after pollination the

² GUIGNARD, I., Sur les anthérozoïdes et la double copulation sexual chez les végétaux angiospermes. *Compt. Rend.* 128: 864-871. 1899.

endosperm cells collapsed and formed a dark staining jacket around the embryo (fig. 43). Others at 6 weeks were like those described at 26 days. The extreme difference at 6 weeks between the endosperms of cross and self pollinated material is indicated by figs. 19 and 20.

4. DEVELOPMENT OF EMBRYO.—The material which yielded sections showing the second male nucleus in contact with the nucleus formed from the fused polars also demonstrated fertilization. Sperms in contact with the egg were seen earliest in material taken 76–78 hours after pollination (figs. 21, 22). This is in general agreement with the fertilization time in selfed *I. versicolor*. In that species the writer¹ reported male nuclei in the embryo sac 72 hours after pollination. Ovaries of this age show some ovules which give no evidence of fertilization, not even of pollen tubes in the micropyle; and other ovules, as has been said, may have two, four, or eight free endosperm nuclei. In crossed material the male nucleus was also seen in contact with the egg as late as 96 hours after pollination (fig. 23). Whether fertilization finally occurs in such cases cannot be stated. No evidence of disorganization at this stage was detected.

The cytology of fertilization is still under investigation. At present the writer would only call attention to the fact that a section of a one celled body in crossed material shows a nucleus with chromosomes in pairs (fig. 24). This arrangement and the number (which is approximately 24) suggest that fusion of the gametes does occur. A corresponding stage has not been seen in self pollinated material. In the crossed material, some of the ovules having gametes in contact still have an undivided primary endosperm nucleus, while others in the same ovary show as many as sixteen free endosperm nuclei. It is apparent that the fusion of the gametes is not so promptly consummated as is the fusion which completes the primary endosperm nucleus.

One- and two-celled embryo stages are found in crossed material, taken 6 days after pollination. The one-celled embryos differ from each other in appearance, as do those of two cells. As one-celled bodies persist in ovules at 7 (fig. 25), 9 (fig. 26), 11 (fig. 27), and 13 days (fig. 28) after pollination, occurring in the same ovaries as

¹ SAWYER, M. LOUISE, Pollen tube and spermatogenesis in *Iris*. BOT. GAZ. 64:159-164. 1917.

ovules with embryos of several cells, these differences are probably related to the differing destinies of these structures believed to be one-celled embryos. Among them some are found in which it is possible to recognize signs of disorganization. In such cases the nuclei are irregular in outline, as if the nuclear membrane were dissolving, and the rather structureless mass tends to stain heavily (figs. 26, 29).

In self pollinated material the two-celled embryo was not found in collections made earlier than the seventh day after pollination. At this time these ovules have a well developed layer of free endosperm nuclei. This may be one of the significant factors in the subsequent history. In the selfed ovules the embryos soon overcome the slight handicap of a day's delay at the start, and for a time their development runs parallel with the more vigorous embryos in ovules of the crossed material. In both types of material, successive cell divisions result in a row of three (fig. 30) or four (fig. 31) cells, after which the end cell undergoes a longitudinal division (figs. 32, 33). A second longitudinal division may occur, as is shown by the fact that a transverse section of the end of the embryo at this stage shows four cells (fig. 34). Elongation of the embryo may continue (fig. 35), or by divisions in the lower cells it may become more massive (figs. 36, 37, 39).

In self pollinated material the development of the embryo then begins to forge ahead at such a rate that the embryo is fully formed and the ovule has attained the ultimate size of the seed at the end of 6 weeks. In ovules of cross pollinated material the history is quite different. Death of the embryo may occur at any stage. A dying embryo 6 days after pollination is shown in fig. 29; others at 11 days in figs. 40 and 41. In these cases the endosperm was still in the free nuclear stage. Twenty-six days after pollination some of the ovules in ovaries that are still living have so shriveled as to nearly obliterate the almost empty embryo sac cavity. Others less withered are partly filled with endosperm, which may be still nucleated, or its cells may have lost their contents. An embryo of this age is shown in fig. 42 with its cells plasmolyzed and distorted. The endosperm surrounding it has reached the pithlike stage (fig. 18). Crossed material which was killed 6 weeks after cross pollination yielded some interesting sections. Most of the ovules were so hardened and shriveled that they infiltrated poorly, and usually no sign of the embryo could be

detected among the fragments of the sectioned ovules; perhaps they would not have been recognizable in any case. In one of the more favorable sections of a less distorted ovule a strand of contracted endosperm cells inclosed a section of the embryo (fig. 43). A few ovules had not hardened their coats. In one of these, which was but little wrinkled, the embryo was found (fig. 44). The endosperm which surrounded it and filled the ovule cavity, however, was pith-like (fig. 19), and it is believed that the embryo could not have lived much longer with so meager a source of food.

This most advanced embryo found in cross pollinated material is but a feeble dwarf, when compared with those of the same age resulting from self pollination. This embryo, consisting of a little mass of undifferentiated cells at the end of a short suspensor, measured $235\ \mu$ in length, $112\ \mu$ in width, and was contained in an ovule which was about $2500\ \mu$ long and $1250\ \mu$ wide. At the same age, that is, 6 weeks after self pollination, those ovules had become seeds 7 mm. in diameter and 3 mm. thick, and contained fully differentiated embryos which were 3.5 mm. long and about 0.3 mm. thick. Figs. 45 and 46, drawn to the same scale, show the relative sizes of the two contrasted embryos. The cells do not differ greatly in size (figs. 47, 48), although the cytoplasm in the crossed embryo was more attenuated, and lacked the metaplasmic bodies similar to those noted in the cytoplasm of the endosperm earlier in this paper. Whatever causes the delay in development, therefore, retards cell division. These embryos never get beyond the proembryo stage.

Conclusions

1. Castrated flowers of *I. Pseudacorus*, covered and not pollinated, drop their ovaries in 4-7 days after being covered.
2. Pollination of *I. Pseudacorus* with *I. versicolor* pollen stimulates the growth of the ovary and prolongs its life.
3. During this investigation such pollination has not resulted in viable seed.
4. Dissection of pollen tubes established the fact that the growth of *I. versicolor* pollen tubes on *I. Pseudacorus* stigmas is able to keep pace with the growth of *I. Pseudacorus* tubes on their own stigmas.
5. Sections show that the time required for pollen tubes to enter the micropyle is essentially the same in cross and self pollinated

ovaries. Tubes were seen in the micropyle of material killed about 77 hours after pollination.

6. The failure to secure viable seed was not due to a failure of the pollen tube to reach the ovules.

7. Free endosperm nuclei appear in both self and cross pollinated specimens shortly after the entrance of the pollen tube in both self and cross pollinated specimens.

8. Male nuclei were observed in contact with the egg in crossed as well as in selfed material.

9. In cross pollinated material the occurrence of the one-celled zygote showing approximately 24 chromosomes is believed to indicate that fusion follows.

10. Following fusion, the history of the zygotes resulting from cross pollination varies widely. Some die without division and death may occur at any subsequent stage.

11. After fertilization, the selfed zygote first lags behind the crossed zygote; then for a short time their development runs parallel; but soon the embryos produced by self pollination develop more rapidly than the crossed embryos. The most advanced embryo resulting from the cross was found in ovules killed 6 weeks after pollination. This embryo is but $2500\ \mu$ long and $1250\ \mu$ wide, while selfed ovules of the same age contain embryos 3.5 mm. long and 0.3 mm. thick, and are fully differentiated.

12. The endosperm in crossed material at 6 weeks after pollination is either a contracted strand or a pithlike tissue of cells without contents, while the endosperm in the selfed material consists of nucleated cells whose cytoplasm is crowded with metaplasmic bodies, food granules.

13. The failure to secure viable seed following cross pollination of *I. Pseudacorus* by *I. versicolor* is due to the death of the embryo at an early stage of development, although it may live long enough to have reached maturity.

The writer gratefully acknowledges the helpful suggestions and criticisms of Professor CHARLES J. CHAMBERLAIN during the progress of this work.

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EXPLANATION OF PLATES V-X

Abbreviations: *a*, pollen tube; *b*, male nucleus; *c*, second male nucleus; *d*, egg nucleus; *e*, synergids; *f*, polar nucleus; *g*, primary endosperm nucleus; *h*, free endosperm nucleus; *i*, antipodal nucleus; *j*, antipodal cell; *k*, embryo; *l*, metaplastic bodies; *m*, endosperm.

PLATE V

FIG. 2.—Transverse section of ovary 6 weeks after pollination; three re-entrant angles separate locules, which are lens shaped in section; $\times 2$.

FIG. 3.—Transverse section of ovary 6 weeks after self pollination; $\times 2$.

FIG. 4.—Micropylar end of ovule 78 hours after self pollination, showing uncollapsed pollen tube which ends in three branches at nucellus; $\times 895$.

FIG. 5.—Micropylar end of ovule 6 days after cross pollination; pollen tube in micropyle; remnant of synergid, zygote, and free endosperm nuclei in embryo sac; $\times 540$.

FIG. 6.—Micropylar end of ovule 77 days after cross pollination; pollen tube forked on reaching nucellus; $\times 895$.

PLATE VI

FIG. 7.—Embryo sac from flower at age when buds were opened for removing anthers; egg apparatus developed and polar nuclei approaching, but antipodal cells not yet organized; $\times 1610$.

FIG. 8.—Antipodal end of embryo sac 12 hours after pollination; antipodal cells organized but polar cells not yet in contact $\times 1610$.

FIG. 9.—Polar nuclei in contact 6 hours after pollination; $\times 980$.

FIG. 10.—Polar nuclei in contact in cross pollinated material 54 hours after pollination; $\times 980$.

FIG. 11.—Egg apparatus still intact 54 hours after pollination; $\times 2540$.

FIG. 12.—Mature embryo sac; nucleus formed by fusion of polars shows two nucleoli; $\times 2540$.

FIG. 13.—Nucleus formed by fusion of polars, showing nucleoli in contact as though fusing; $\times 1610$.

FIG. 14.—Antipodal end of embryo sac with two antipodal cells and second male nucleus in contact, with nucleus formed by fusion of polars; 77 hours after cross pollination; $\times 2540$.

PLATE VII

FIG. 15.—Male nucleus in contact with nucleus resulting from fusion of polars, 78 hours after self pollination; $\times 2540$.

FIG. 16.—Free endosperm nuclei 11 days after cross pollination; $\times 1610$.

FIG. 17.—Cells of endosperm tissue 20 days after cross pollination; $\times 895$.

FIG. 18.—Cells of endosperm tissue 26 days after cross pollination; cells have lost their contents; $\times 895$.

FIG. 19.—Cells of endosperm tissue 6 weeks after cross pollination; $\times 895$.

FIG. 20.—Cells of endosperm tissue 6 weeks after self pollination; $\times 895$.

FIG. 21.—Fertilization 77 hours after cross pollination; pollen tube extends well into embryo sac; $\times 2540$.

PLATE VIII

FIG. 22.—Fertilization 78 hours after self pollination; free endosperm nucleus shown lying in embryo sac cytoplasm which is filled with metaplasmic bodies; expanded end of pollen tube lies within embryo sac; $\times 2540$.

FIG. 23.—Male nucleus in contact with egg 96 hours after cross pollination; $\times 2540$.

FIG. 24.—Nucleus of zygote 6 days after pollination, with approximately 24 chromosomes mostly arranged in pairs; $\times 2540$.

FIG. 25.—Zygote 7 days after cross pollination; $\times 2540$.

FIG. 26.—Zygote 9 days after cross pollination; $\times 2540$.

FIG. 27.—Zygote 11 days after cross pollination; $\times 2540$.

FIG. 28.—Zygote 13 days after cross pollination; $\times 2540$.

FIG. 29.—Two-celled embryo beginning to disorganize 6 days after pollination; membrane of inner cell broken down; $\times 2540$.

FIG. 30.—Embryo 9 days after self pollination; $\times 980$.

FIG. 31.—Embryo 9 days after cross pollination; $\times 980$.

FIG. 32.—Embryo 9 days after cross pollination, having vertical division of terminal cell; $\times 980$.

PLATE IX

FIG. 33.—Embryo in which vertical division occurred in fourth cell; $\times 980$.

FIG. 34.—Transverse section of tip of embryo, showing 4 cells; $\times 2540$.

FIG. 35.—Elongated embryo 11 days after cross pollination; $\times 540$.

FIG. 36.—Massive embryo 9 days after cross pollination; $\times 540$.

FIG. 37.—Massive embryo 11 days after self pollination; $\times 980$.

FIG. 38.—Two-celled embryo 6 days after cross pollination; $\times 980$.

FIG. 39.—Massive embryo 13 days after cross pollination; $\times 980$.

FIG. 40.—Embryo beginning to die; nuclei of some cells disorganized (11 days after cross pollination); $\times 980$.

FIG. 41.—Embryo dying; cells contracted and formless (11 days after cross pollination); $\times 980$.

PLATE X

FIG. 42.—Embryo 26 days after cross pollination; $\times 980$.

FIG. 43.—Strands of contracted endosperm inclosing embryo, 26 days after cross pollination; $\times 895$.

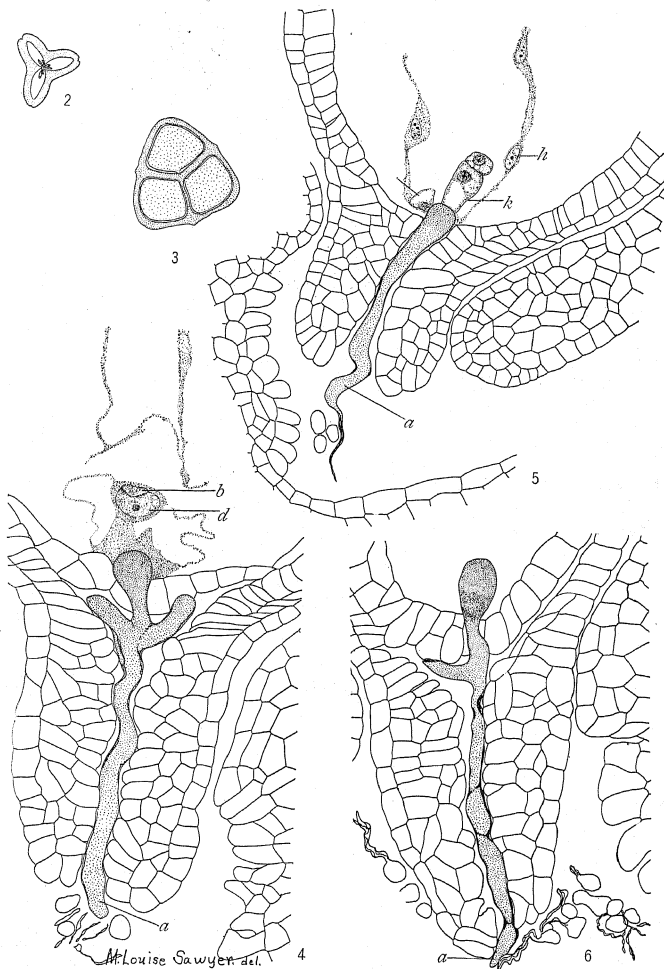
FIG. 44.—Embryo 6 weeks after cross pollination; $\times 895$.

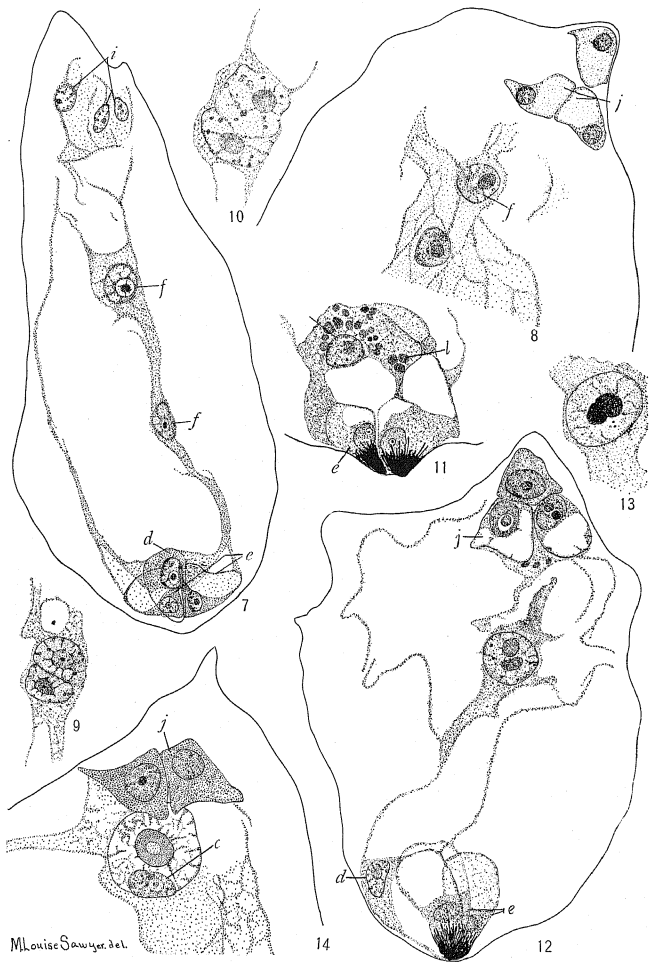
FIG. 45.—Embryo 6 weeks after cross pollination (same embryo as in fig. 44 drawn to same scale as next figure, for purpose of comparison).

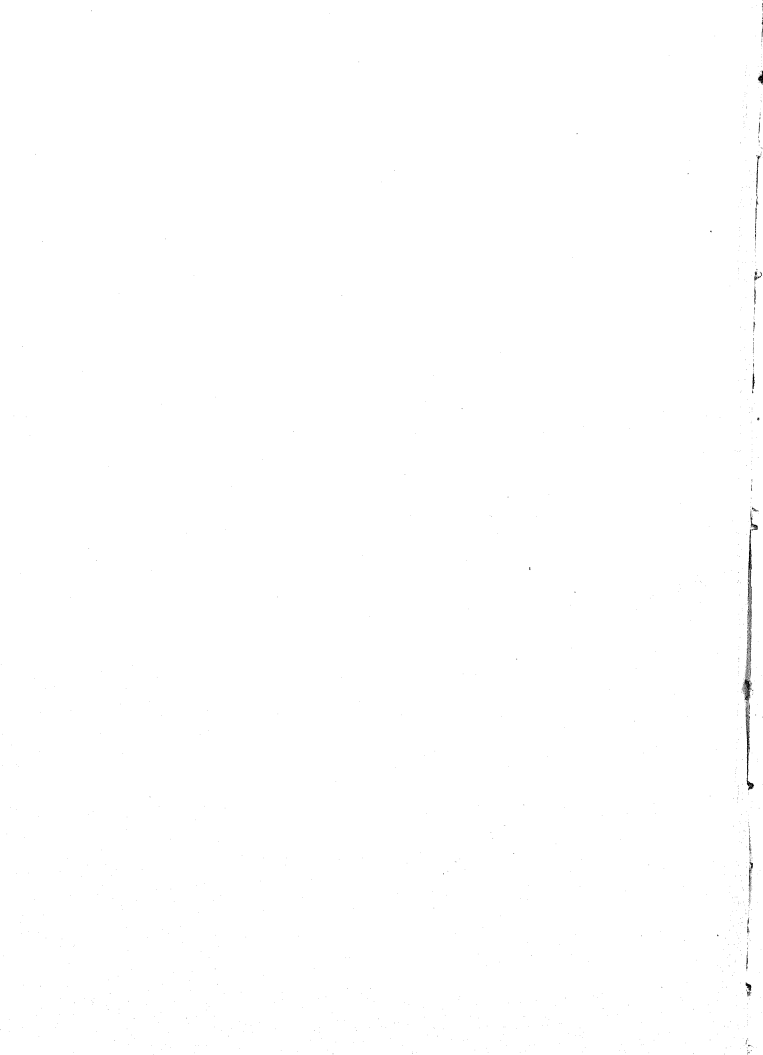
FIG. 46.—Embryo 6 weeks after self pollination (drawn to same scale as fig. 45).

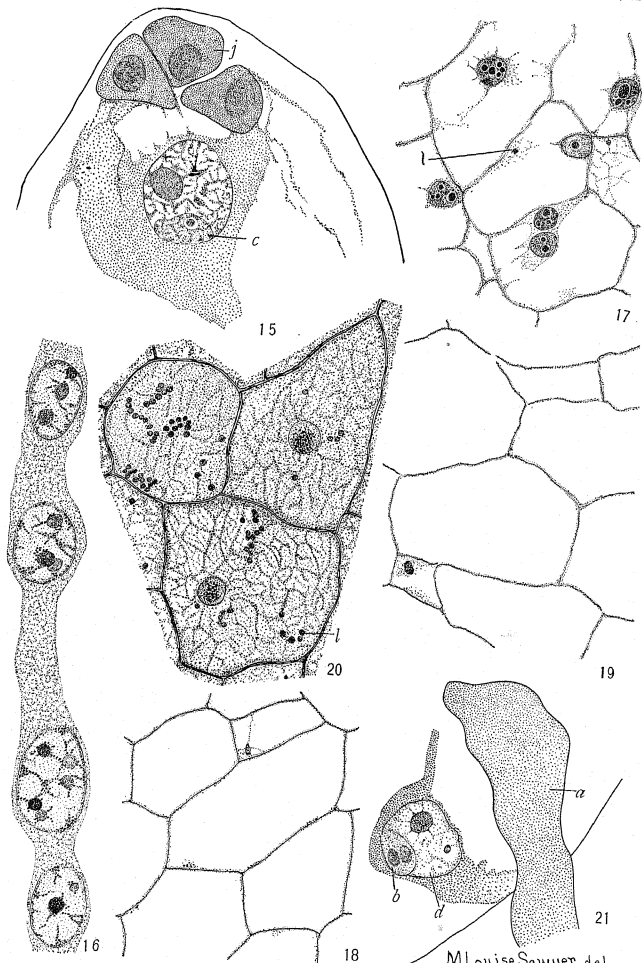
FIG. 47.—Group of cells from embryo 6 weeks after cross pollination; $\times 1610$.

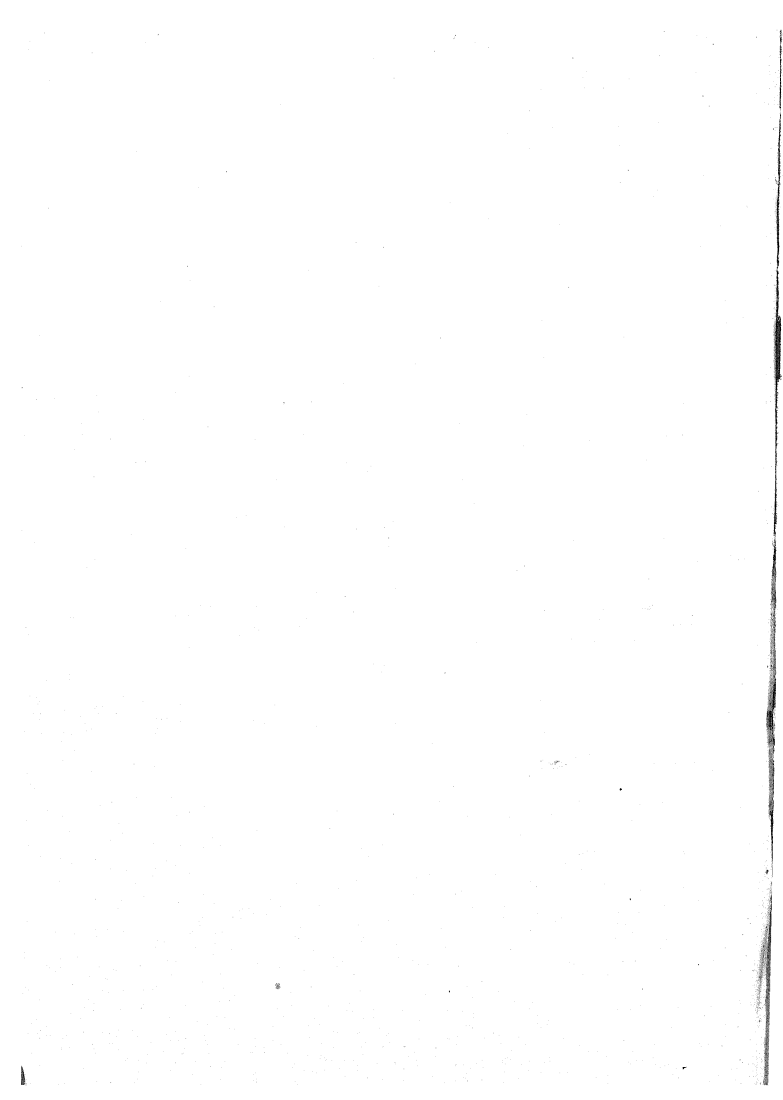
FIG. 48.—Group of cells from embryo 6 weeks after self pollination; $\times 1610$.





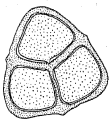




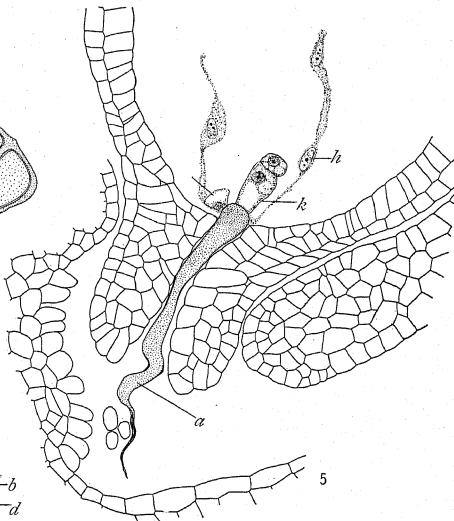




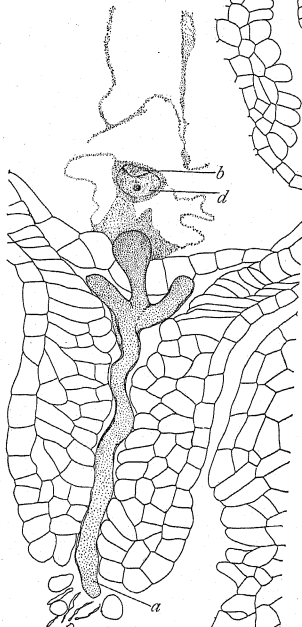
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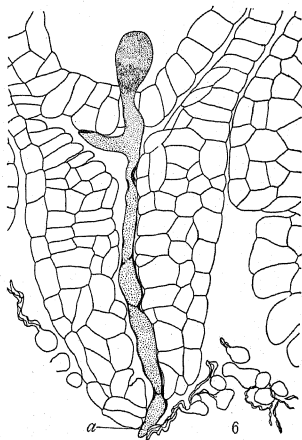
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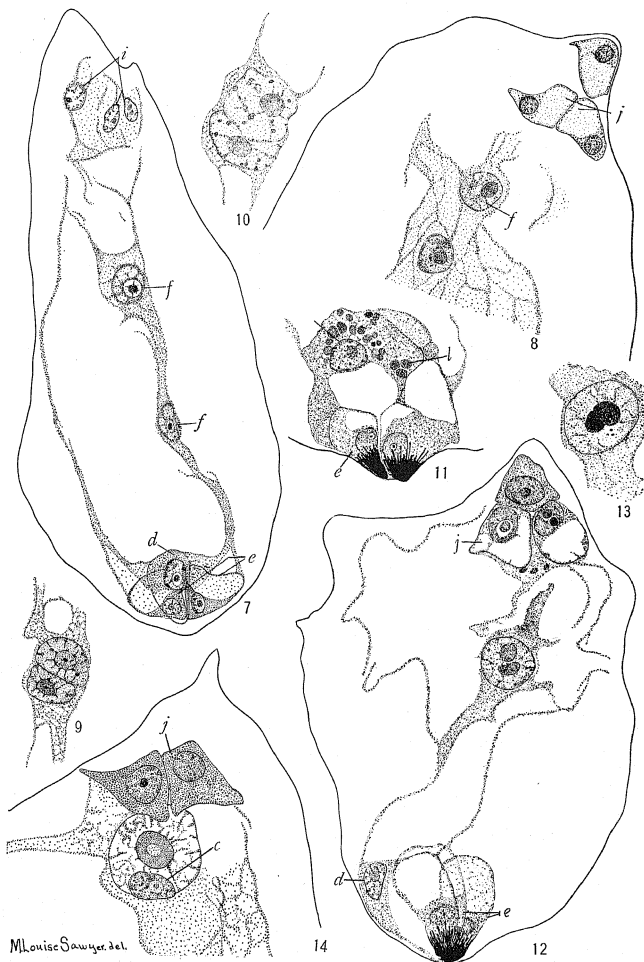
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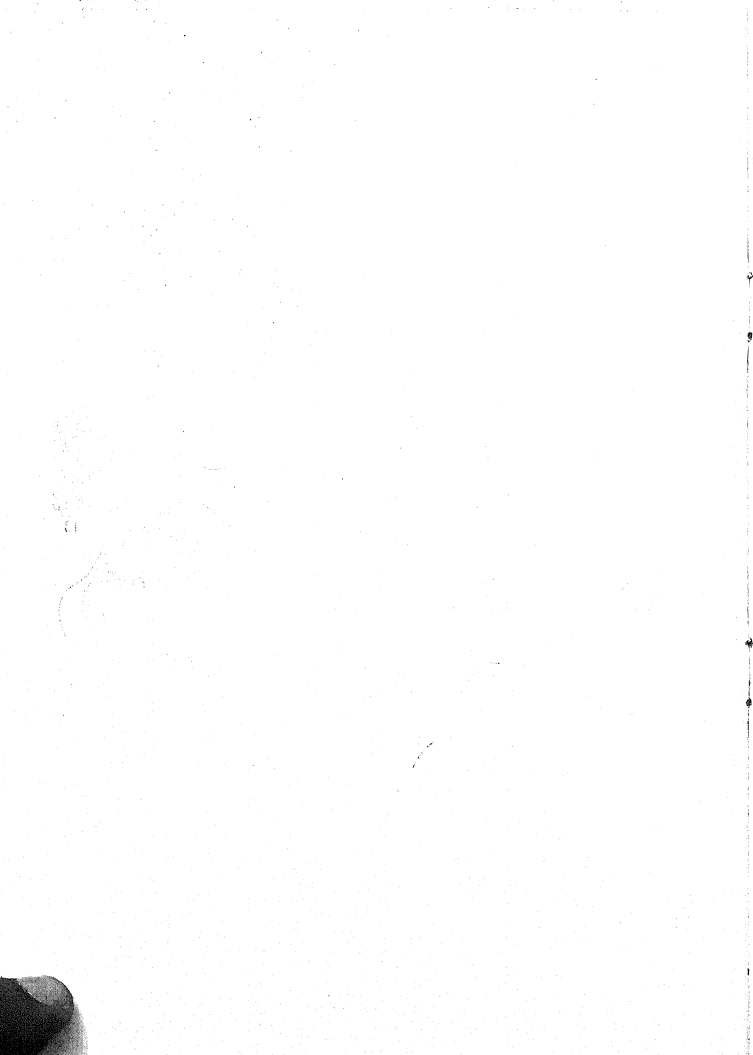
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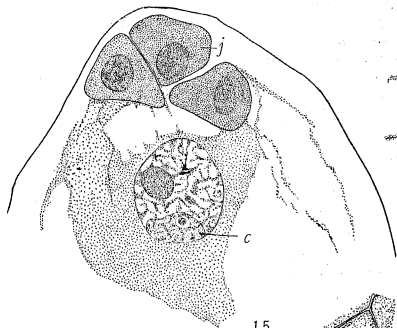
M. Louise Sawyer del.



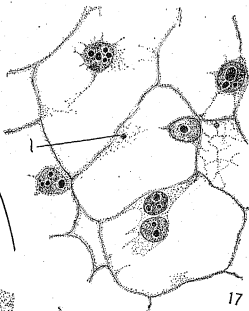


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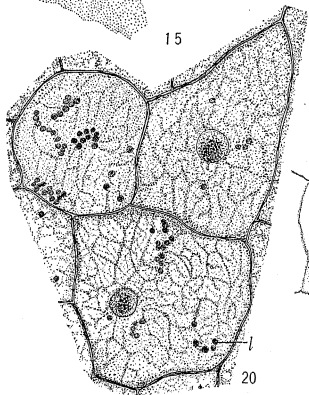




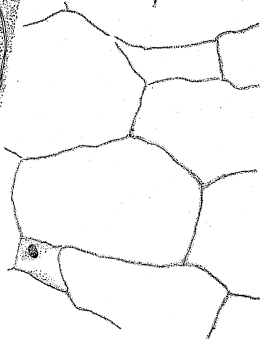
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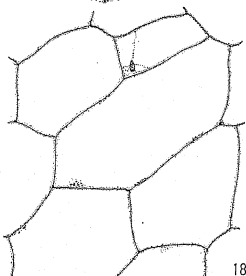
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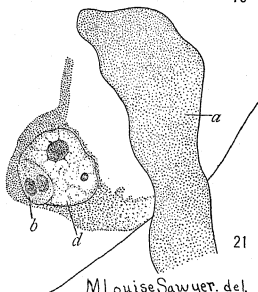
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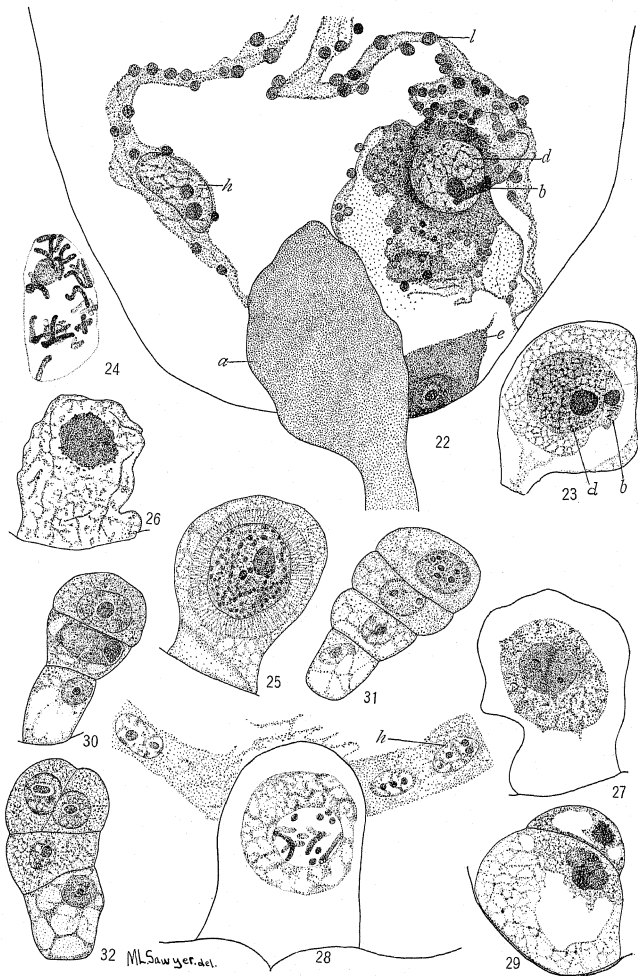


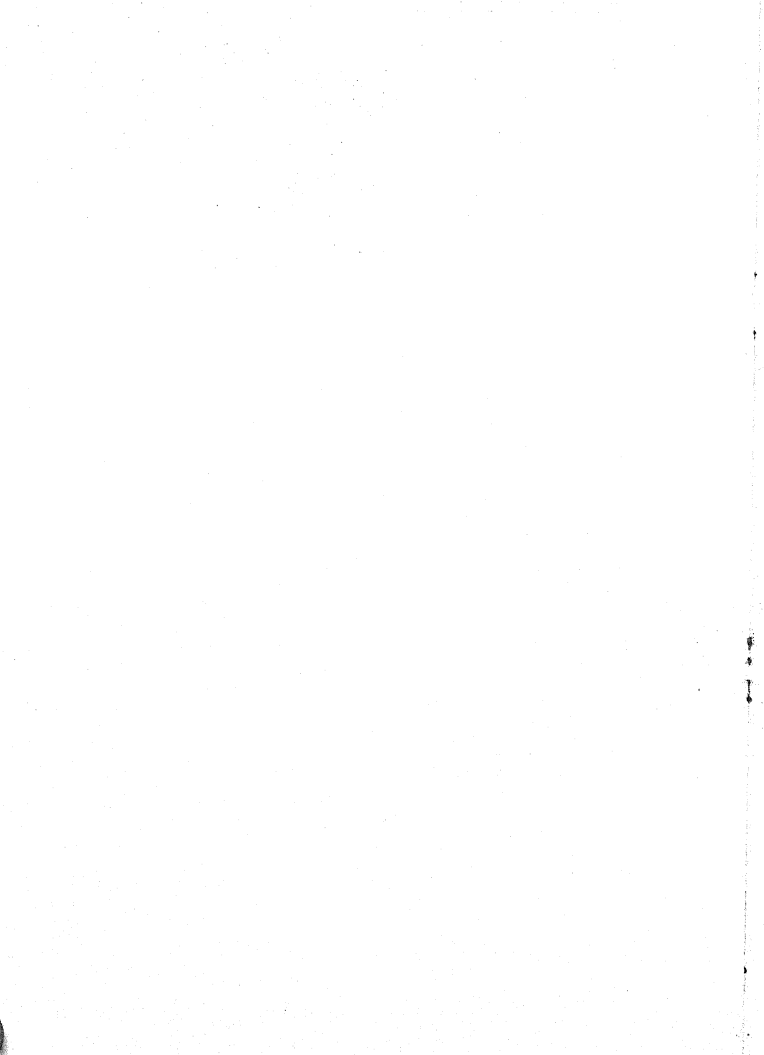
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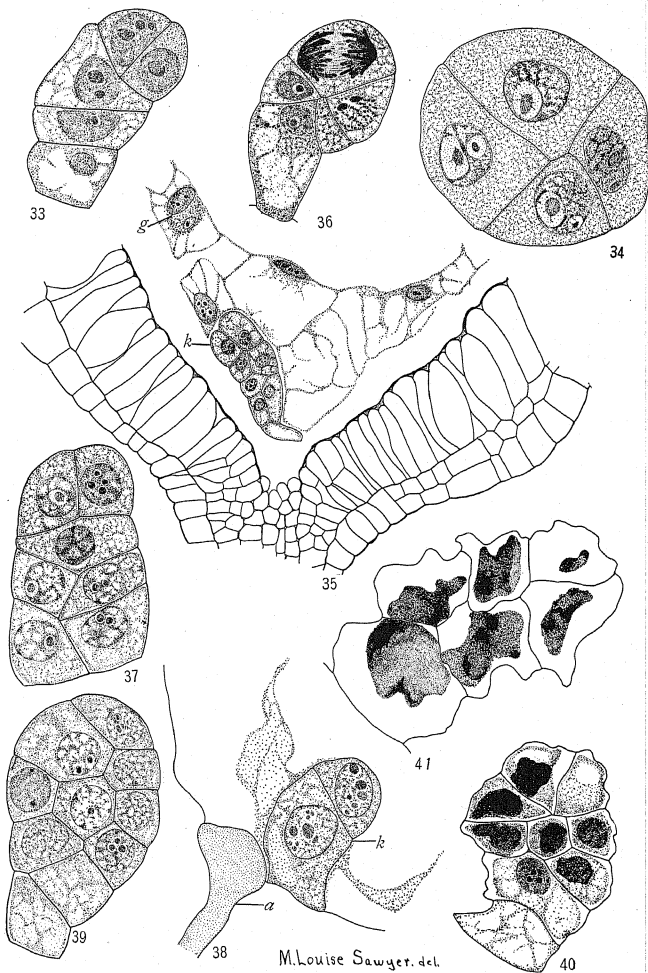


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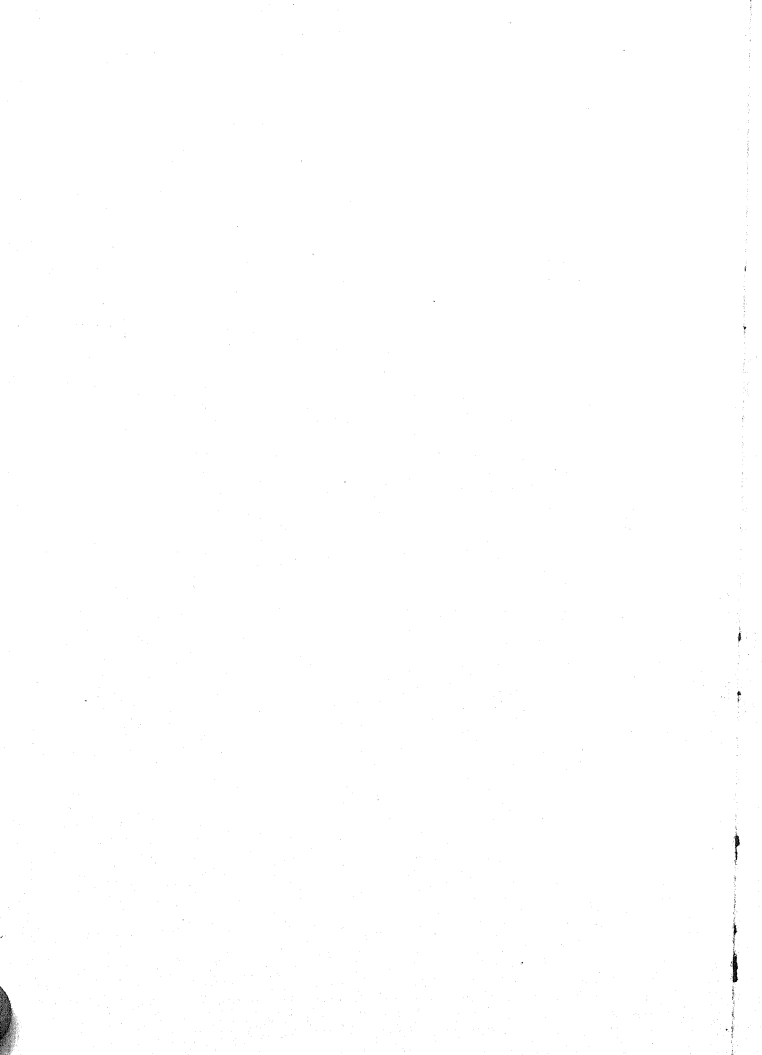
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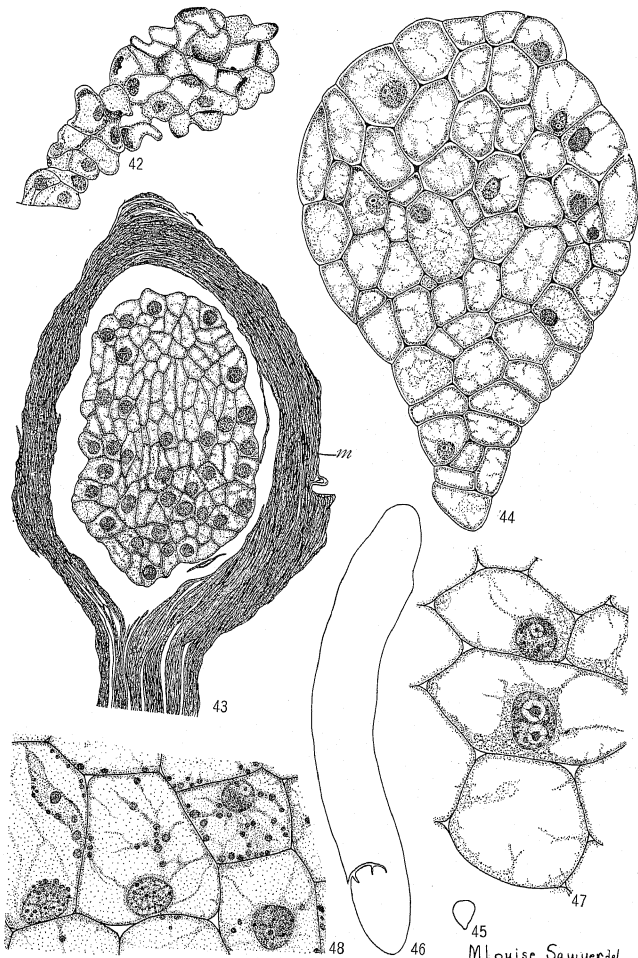






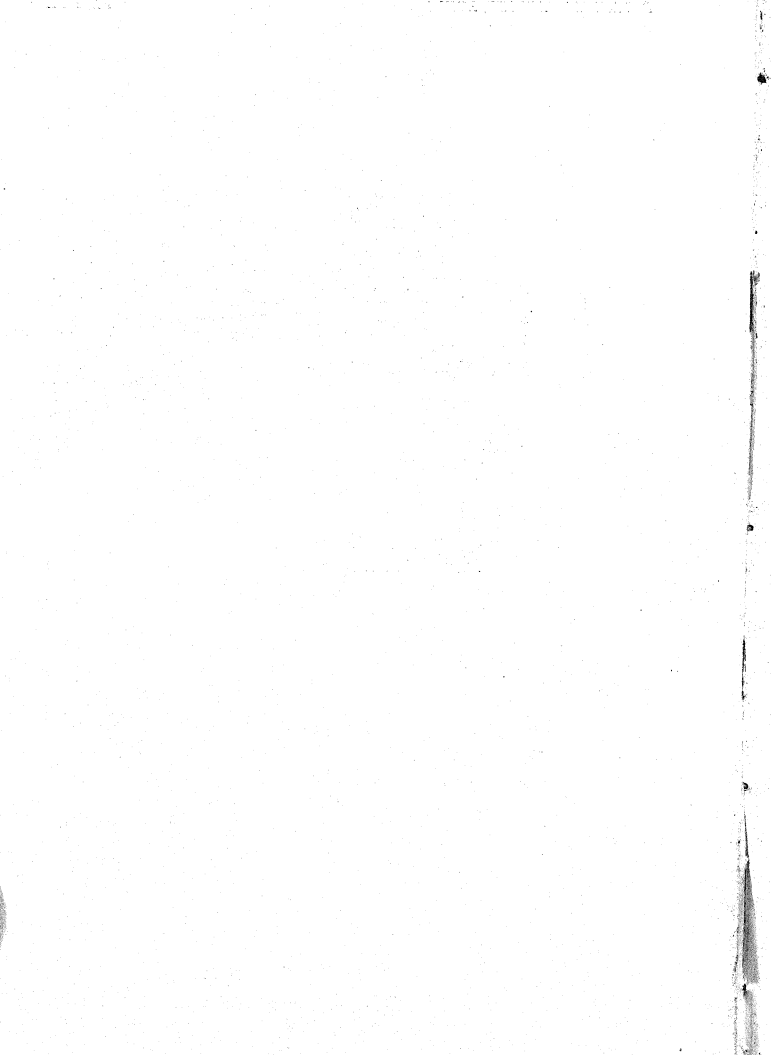
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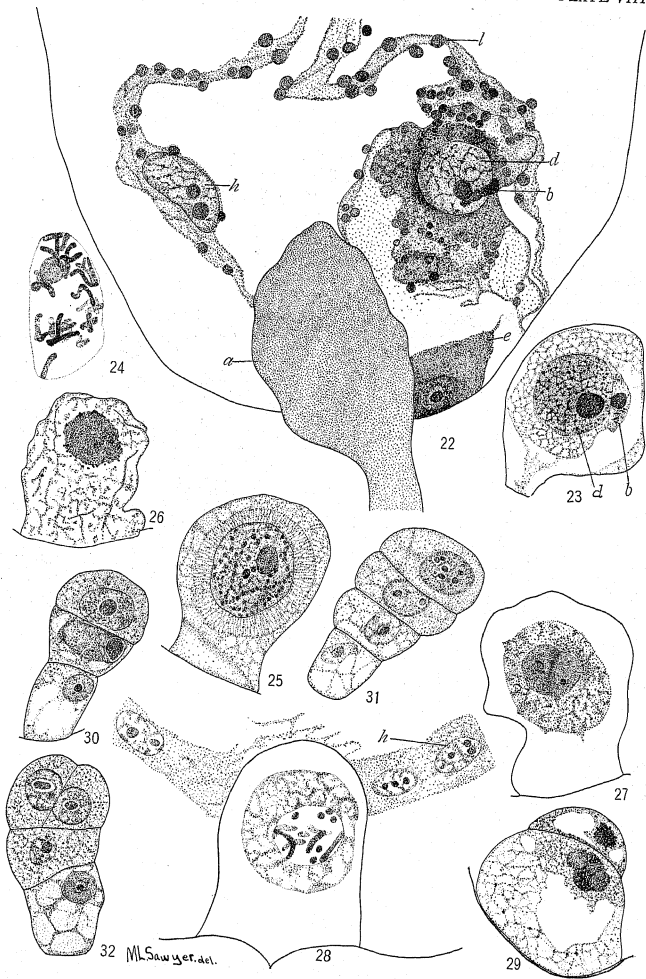




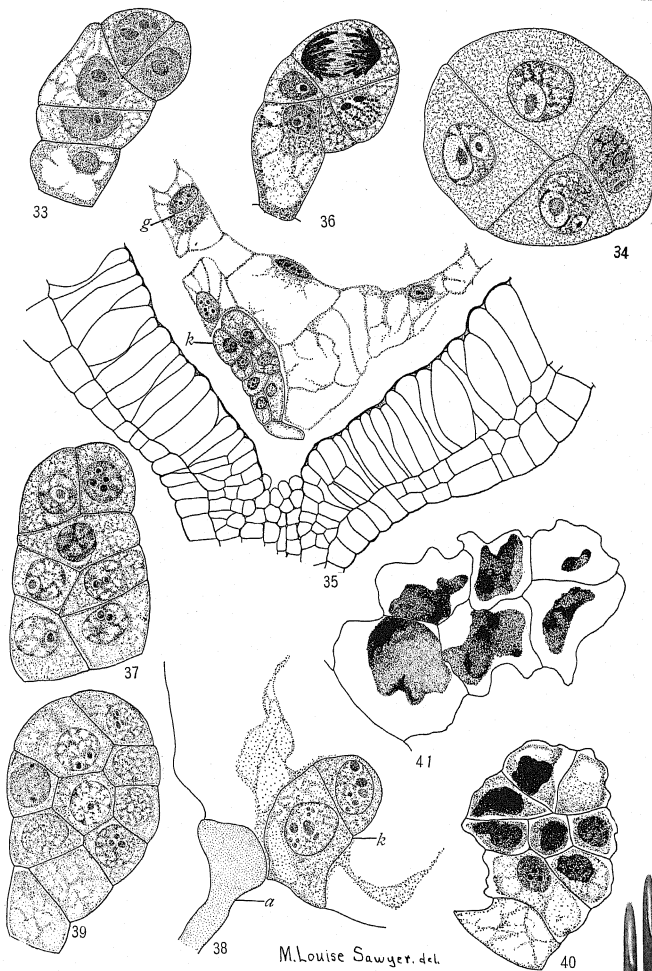
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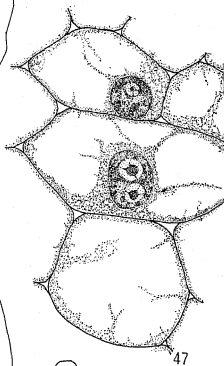
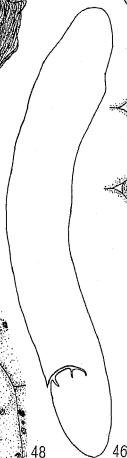
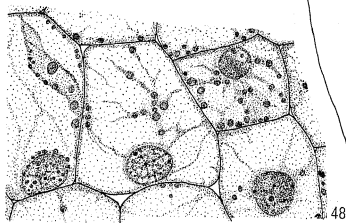
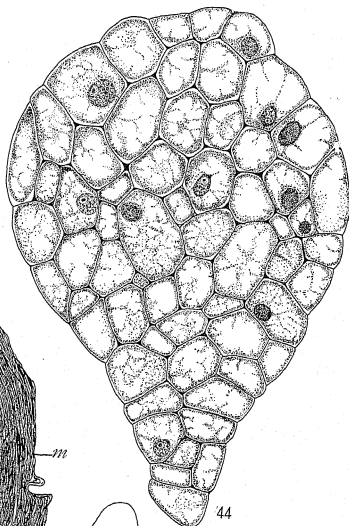
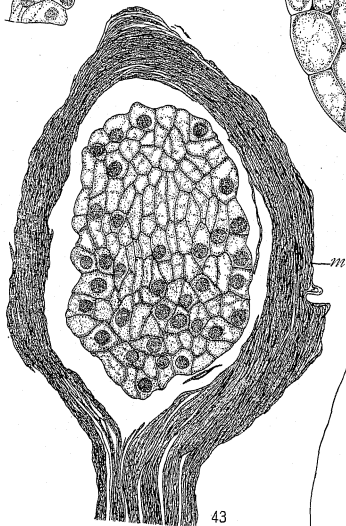
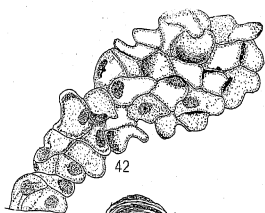




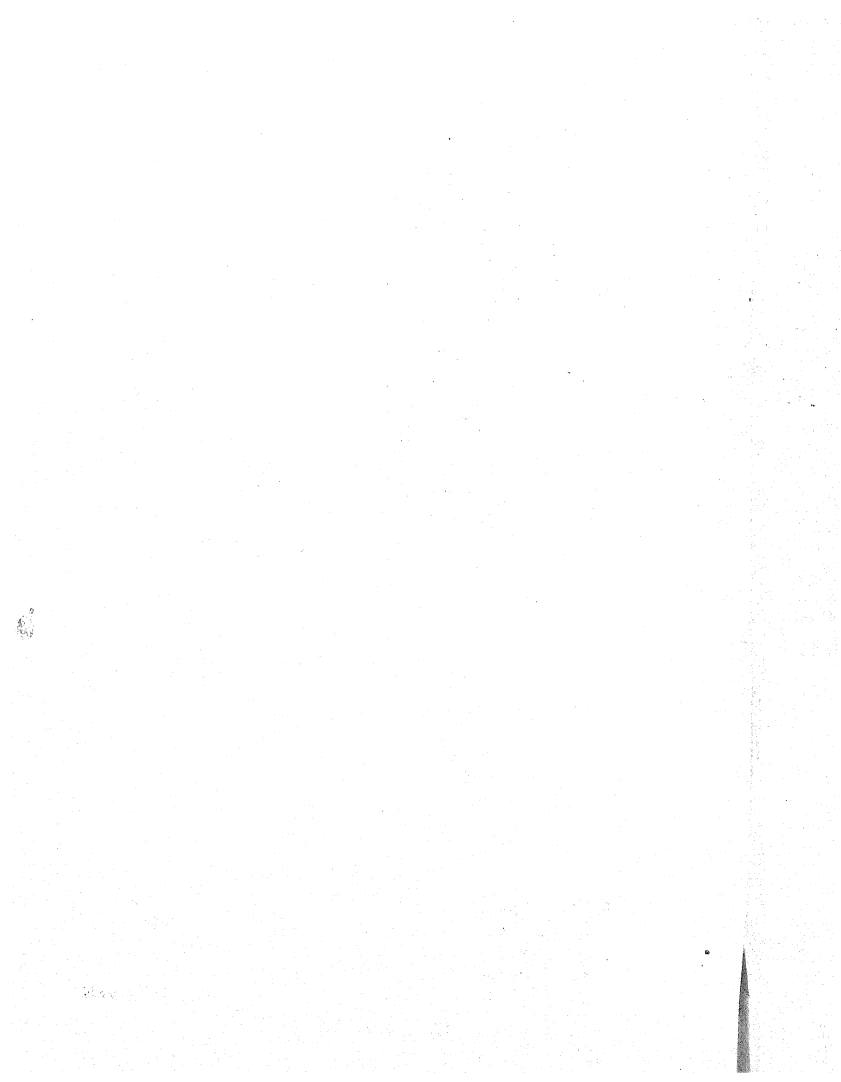


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CATALASE ACTIVITY AND THE AEROBIC AND ANAEROBIC GERMINATION OF RICE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 331

TOSHITARO MORINAGA

Introduction

The relation of anaerobiosis and catalase activity has been studied by a number of workers, with somewhat conflicting results. According to RYWOSCH and RYWOSCH (13) and SPIRO (16), although aerobic bacteria contain much catalase, the anaerobic bacteria possess practically no catalase activity. The same claim has been made recently by McLEOD and GORDON (10), and also by CALLOW (4). Some years ago LESSER (7, 8) showed that the blood of poikilothermous animals contains much less catalase than that of mammals. Among the animals which he investigated, *Ascaris* showed the least catalase activity. On the other hand, LOEW (9) found that yeasts and the bacillus of black-leg, an obligate anaerobe, possessed catalase activity, and stated that catalase might be an agent for fermentative as well as respiratory phenomena. Studying zymine in wheat seedlings, PREOBRASCHENSKY (12) found that substances which promote alcoholic fermentation also increase the amount of catalase, while inhibitors of fermentation also depress the catalase activity of the yeast. His conclusion was that catalase may take part in anaerobic processes. Notwithstanding the numerous contributions which have been made in recent years by both animal and plant physiologists, the physiological meaning of catalase activity is still very obscure, and we are not certain what part if any it plays in the dynamics of cell life, or whether it is connected with fermentative or respiratory processes.

The work reported here was undertaken to determine the influence of anaerobic and aerobic conditions of germination upon the development of catalase activity in seedlings of some of the higher green plants, with the hope that it might throw some light on the relation of catalase to the dynamic cell processes.

Materials and methods

A number of the grains were chosen for this work, including rice, because the latter, as has long been known, can germinate normally under water, or even in complete absence of oxygen. For comparison, seeds of barley, wheat, oats, and rye, which cannot be germinated so successfully in absence of oxygen, were used. Two varieties of rice, Ishijiro and Kagamochi, were obtained from Japan, and two others, Honduras and Acadia, from the Rice Experiment Station of Louisiana, Marquis spring wheat and seeds of barley and oats were supplied by the Wisconsin Agricultural Experiment Station. The rye was purchased from the Vaughan Seed Company of Chicago. All of these seeds were supposed to have been harvested in 1922. The viability tests showed that all of them, but particularly the varieties of rice, still retained high germinating power. The writer wishes to thank those who assisted by sending seeds of known age.

For the determination of catalase activity, a modified Appleman apparatus (2) was used, and the liberation of oxygen from hydrogen peroxide was used as a measure of the activity. The method was similar to that used by CROCKER and HARRINGTON (6). The Oakland Chemical Company "dioxogen" was used, the concentration of the H_2O_2 being determined for each bottle by permanganate titration, and the equivalent of 10 cc. taken for each determination. The acidity was neutralized by the addition of N/10 NaOH just before using.

Dry seeds were pulverized in a mortar and sifted through a 100 mesh sieve. Any portions not passing through the sieve were re-ground and sifted until there was no remaining part. The powder was thoroughly mixed in the mortar again, and samples weighed out from the mixed materials. When wet determinations were made, the materials were ground with sand and $CaCO_3$ in a mortar for five minutes. In the case of either wet or dry determinations, the material was put into the shaker bottle, with 25 cc. of distilled water, and allowed to reach the temperature of the bath. Then the equivalent of 10 cc. of the peroxide previously diluted to 25 cc. was added, after which the shaker was run at the rate of 100 times per minute, in a bath kept at 21° C. This temperature was adopted because it could easily be maintained; but catalase, like many other enzymes, is most active at about 35° C.

To determine the respiration occurring during germination when such tests were run, a bottle of known internal volume, containing a seed holder and a small NaOH receiver, was connected to a manometer. Several bottles of this type were used in a Freas thermostat at 25° C. The oxygen consumption was calculated from the reduction of manometric pressure, after the manner of SHERMAN (14), while the CO₂ released was estimated from the NaOH in the respirometer by the double titration method of BROWN and ESCOMBE (3). Blank experiments were also run to check the results.

Experimentation

1. COMPARATIVE CATALASE ACTIVITY OF AIR DRIED GRAINS.—To determine the catalase activity of the ungerminated dry seeds, 0.2 gm. of the dry powder of each kind of seed was taken. Table I shows the results for all the varieties of seeds used, including the four varieties of rice. It is seen that the rice varieties stand in contrast to the other grains in catalase activity of the dry, ungerminated seeds.

TABLE I
CATALASE ACTIVITY OF 0.2 GM.
DRY SEED POWDER

SEED USED	OXYGEN LIBERATED IN CC. AFTER	
	6 minutes	16 minutes
Wheat.....	4.0	8.3
Oats.....	5.2	11.9
Barley.....	4.0	9.5
Rye.....	9.9	16.3
Rice (Ishijiro).....	0.4	1.1
(Honduras).....	0.2	0.6
(Acadia).....	0.6	1.1
(Kagamocho).....	0.5	1.4

2. COMPARATIVE CATALASE ACTIVITY IN AEROBICALLY GERMINATED SEEDS.—In this experiment, grains of oats, wheat, barley, and rice were germinated on moist filters in Petri dishes. One group of seeds was kept at about 25° C., floating on the water bath, and another at 16° C. in an icebox. The seeds were tested at equal stages in germination, the rice requiring considerably longer time to reach a given stage of development. Much more time was required at the lower temperature than at the higher one, for a particular stage to

be reached. Ten of the seeds were used for each determination. The results are shown in table II.

3. CATALASE ACTIVITY IN ANAEROBICALLY GERMINATED RICE.—The rice was germinated in thoroughly boiled distilled water, protected from contact with air by a mercury seal. This method was checked by germinating the seeds in purified hydrogen gas. The rice (Ishijiro) germinated under these conditions in two days, although it did not develop roots. The samples, ten grains or seedlings of each,

TABLE II
CATALASE ACTIVITY OF AEROBICALLY GERMINATED SEEDS;
OXYGEN IN CC. AT STANDARD CONDITIONS

MATERIAL	HOURS GERMINATED	TEMPERATURE (° C.)	OXYGEN LIBERATED	
			6 minutes	12 minutes
Oats.....	18	25	13.5	23.1
Wheat.....	24	25	18.4	27.0
Barley.....	24	25	14.9	23.3
Rice (Ishijiro).....	54	25	10.8	18.2
Oats.....	41	16	15.6	24.6
Wheat.....	46	16	15.4	14.0
Barley.....	48	16	12.6	21.7
Rice (Ishijiro).....	258	16	9.6	16.8

were taken from the germinators each day for catalase tests. Comparative tests were made on grains growing under aerobic conditions on wet filter papers, and with seeds growing under 7.7 cm. of water, unboiled and unsealed. Note was made of the average state of development of plumule and radicle under the conditions employed. The results of these experiments are given in table III.

4. CATALASE ACTIVITY OF RICE GERMINATED AT REDUCED OXYGEN PRESSURES.—In this experiment the rice was germinated under reduced atmospheric pressure, as follows: $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$ atmospheres. The samples, ten grains or seedlings of each, were taken each day for a catalase determination. The results are shown in table IV.

The reduction of catalase activity is very marked as the oxygen pressure is reduced.

5. CATALASE ACTIVITY OF RICE GERMINATED UNDER WATER AT DIFFERENT DEPTHS.—Ordinary water of 2, 4, 6, 8, 15, and 30 mm. depth was kept in Petri dishes and beakers in which Kagamochi

TABLE III
CATALASE ACTIVITY OF RICE GROWN ANAEROBICALLY;
OXYGEN IN CC. AT STANDARD CONDITIONS

CONDITION OF GERMINATION	TIME IN HOURS	OXYGEN LIBERATED		DEVELOPMENT IN MM.	
		6 minutes	12 minutes	Plumule	Radicle
Wet filters	24.....	2.7	4.5	0	0
	48.....	12.6	19.7	Starting	2.0
	72.....	21.9	32.2	2.8	9.8
	96.....	35.2	46.7	5.2	22.9
Under water 7.7 cm.....	24.....	1.1	2.4	0	0
	48.....	2.9	5.4	2.0	0
	72.....	5.3	9.5	10.0	Starting
	96.....	8.2	12.9	19.0	3.0
In hydrogen gas	24.....	1.1	2.0	0	0
	48.....	1.4	3.0	1.0	0
	72.....	2.1	4.2	4.8	0
	96.....	2.5	4.6	15.3	0
In boiled distilled water under seal..	24.....	0.9	2.2	0	0
	48.....	1.1	2.0	1.0	0
	72.....	1.4	3.2	6.0	0
	96.....	1.4	2.6	9.0	0

TABLE IV
REDUCED OXYGEN PRESSURE AND CATALASE; GERMINATION
AT ROOM TEMPERATURE

ATMOSPHERIC CONDITION	HOURS OF GERMINATION	OXYGEN LIBERATED		DEVELOPMENTAL STAGE, IN MM.	
		6 minutes	12 minutes	Plumule	Radicle
1/2.....	24.....	2.0	4.3	0	0
	48.....	8.5	13.7	Starting	Starting
	72.....	22.7	33.2	1.4	6.4
	96.....	33.7	47.0	5.6	19.6
1/4.....	24.....	0.9	2.5	0	0
	48.....	4.9	10.0	Starting	Starting
	72.....	12.5	21.4	1.5	3.9
	96.....	31.9	46.5	20.1	21.6
1/8.....	24.....	1.1	2.4	0	0
	48.....	3.0	5.9	Starting	Starting
	72.....	7.2	13.0	2.5	2.2
	96.....	13.4	22.6	6.2	11.1
1/16.....	24.....	1.1	2.1	0	0
	48.....	1.7	3.5	Starting	Starting
	72.....	3.2	6.4	4.7	0.8
	96.....	5.1	10.5	10.8	4.3

rice was germinated. After six days of submerged germination and growth, ten seedlings of each were taken as samples for the determinations. The results are tabulated in table V.

TABLE V
SUBMERGENCE AND CATALASE ACTIVITY OF RICE;
GROWN AT ROOM TEMPERATURE

CONDITION	OXYGEN LIBERATED		STAGE OF DEVELOPMENT		RATIO, PLUMULE RADICLE
	3 minutes	6 minutes	Plumule	Radicle	
On wet paper.....	33.9	46.8	7.8	16.0	0.48
In 2 mm. H ₂ O.....	28.9	40.8	9.5	21.0	0.45
In 4 mm. H ₂ O.....	26.1	37.7	10.5	22.0	0.47
In 5 mm. H ₂ O.....	16.3	26.8	18.0	6.8	2.64
In 8 mm. H ₂ O.....	14.7	23.0	17.8	4.3	4.13
In 15 mm. H ₂ O.....	10.8	17.4	13.2	3.3	4.00
In 30 mm. H ₂ O.....	10.4	16.9	14.0	4.0	3.50

About the same results were obtained with Ishijiro. The reduction of catalase activity of seedlings grown under water is very marked as the depth increases. The radicle development falls off rapidly with increasing depth of submergence.

TABLE VI
SUBMERGENCE AND CATALASE ACTIVITY OF WHEAT AND
BARLEY; GROWN AT ROOM TEMPERATURE

PLANT	CONDITION	LENGTH OF PLUMULE (mm.)	OXYGEN LIBERATED	
			2 minutes	4 minutes
Wheat	On wet paper.....	29	47.5	68.4
	In 2 mm. H ₂ O.....	30	42.4	64.9
	In 4 mm. H ₂ O.....	20	26.3	49.7
	In 6 mm. H ₂ O.....	0	4.1	8.1
	In 8 mm. H ₂ O.....	0	3.0	5.8
Barley	On wet paper.....	26	37.8	61.3
	In 2 mm. H ₂ O.....	27	25.9	43.7
	In 4 mm. H ₂ O.....	19	24.3	42.0
	In 6 mm. H ₂ O.....	0	2.4	6.8
	In 8 mm. H ₂ O.....	0	2.6	6.3

6. CATALASE ACTIVITY OF WHEAT AND BARLEY GERMINATED IN SHALLOW WATER AT DIFFERENT DEPTHS.—These experiments were conducted as were those upon rice. After four days five seedlings or grains were taken for a determination, with the results shown in table VI.

The decrease in catalase activity is very great between 4 and 6 mm. in depth.

7. DECREASE OF CATALASE ACTIVITY UNDER ANAEROBIC CONDITIONS.—The rice (Ishijiro) was put on moist paper for four days, and then transferred into the bottle containing thoroughly boiled distilled water. The samples, of ten seedlings each, were taken every second day for the catalase tests. The results are tabulated in table VII.

TABLE VII
DECREASING CATALASE ACTIVITY WITH ANAEROBOSIS;
STANDARD CONDITIONS

HOURS UNDER WATER	OXYGEN LIBERATED		GROWTH UNDER WATER (MM.)	
	6 minutes	12 minutes	Plumule	Radicle
0.....	43.1	53.9	0.0	0.0
48.....	30.8	48.8	4.3	2.8
96.....	26.8	42.0	6.6	3.1
144.....	18.3	27.2	12.0	0.5

8. RESPIRATION OF AEROBIC AND ANAEROBIC SEEDLINGS.—In this work forty grains of Ishijiro were kept on moist paper for aerobic respiration, and in boiled distilled water for anaerobic germination. They were allowed to respire under these conditions for two or four

TABLE VIII
RESPIRATION OF AEROBIC AND ANAEROBIC RICE SEEDLINGS;
OXYGEN AND CARBON DIOXIDE IN CC. AT
STANDARD CONDITIONS

Time	Germination method	Oxygen absorbed	Carbon dioxide liberated	$\frac{\text{CO}_2}{\text{O}_2}$	$\frac{\text{O}_2 \text{ aerobic}}{\text{O}_2 \text{ anaerobic}}$
2 days...	Aerobic.....	4.9	5.8	1.17 0.56
	Anaerobic.....	2.8	4.0	1.41	
4 days...	Aerobic.....	9.2	9.4	1.02 0.44
	Anaerobic.....	4.1	4.3	1.05	

days, after which the seedlings were transferred to the respirometers, the condition of temperature having been kept constant at 25° C. throughout the development and respiration tests. The oxygen taken in and the carbon dioxide given off in 20 hours were determined for each. The results are given in table VIII.

Then eighty seedlings, which had been grown under aerobic or anaerobic conditions for 4 days as before, were transferred to bottles in which air had been replaced by hydrogen. The carbon dioxide given off in 20 hours was determined for each, with the results presented in table IX.

TABLE IX
CARBON DIOXIDE LIBERATION BY AEROBIC AND
ANAEROBIC SEEDLINGS IN HYDROGEN

Materials	Aerobic	Anaerobic
Ishijiro rice (80)	10.7	8.4

Discussion

From the first experiments it is clear that the catalase activity of the dry seeds of rice is only about one-tenth of that of barley, wheat, oats, and rye. In practice, rice is germinated under shallow water with a reduced supply of oxygen, and it is known that it can germinate in a medium which contains no free oxygen (11, 17). The same is true also of *Alisma Plantago aquatica* (5). It is considered a general characteristic of seeds which grow in media containing little free oxygen, to be able to germinate without it (15). As mentioned in the introduction, the anaerobic bacteria do not contain any catalase, or quantities so small as to be negligible, and *Ascaris* contains very little. Both live in oxygen free media. Although other experiments are needed on more diverse material to determine the facts, when rice is compared with the other grains, there seems to be some connection between the reduced demand for oxygen for germination and the reduced amount of catalase activity possessed by rice. Experiment no. 2 shows that all the kinds of grain examined except rice possess nearly the same catalase activity when they germinate on wet paper. There is also little difference in catalase activity when the seeds are germinated at different temperatures. The germinating rice contains about seven-tenths the activity of the others, but it was noticed that rice required longer for germination than the other seeds.

From the third set of experiments it is evident that the free oxygen content in the medium in which the rice is germinated affects very remarkably the increase of catalase activity during the course of

germination. In a medium of thoroughly boiled water the activity did not increase; that is, after 24 hours in the boiled water, ten grains liberated 2.21 cc. of oxygen in 12 minutes, whereas after 96 hours they liberated only 2.6 cc. of oxygen in 12 minutes. The average plumule development in this latter case was 9 mm. in length. In a medium of hydrogen gas the catalase activity increased very slightly, but this slight increase was supposed to be due to the insufficient replacement of the air by hydrogen. Under water of 7.7 cm. depth, or on wet paper in atmospheric conditions, the catalase activity increased very rapidly. Thus after 96 hours those in water liberated 12 cc. of oxygen in 12 minutes, and those on filter paper 46.7 cc. In each case ten seedlings were used.

The fourth experiment was carried out to verify by another method the relation between oxygen and catalase already mentioned. Under one-half atmospheric pressure, catalase activity increased rapidly, as under normal pressure, but with one-fourth atmosphere, development of activity was retarded for the first two or three days, although it increased very rapidly after that. Under one-eighth and one-sixteenth atmospheres, the increase of the catalase action was retarded corresponding to the decrease in pressure. As has been observed by others (1, 9, 11), the development of plumule and radicle is readily affected by the amount of free oxygen in the medium. Oxygen retards the elongation of the plumule and accelerates the development of the radicle. The lack of oxygen has exactly the reverse effect.

The data in tables V and VI show more closely the relation between water depth in the germinator and development of catalase activity. A little increase, say 2 mm., clearly affected the catalase development of the seedling. When the depth of water reached 6 mm., which is just enough to cover the whole caryopsis, catalase activity dropped rapidly, and then with increasing depths more slowly. Not only is catalase sensitive to the lack of oxygen, but also the plumule and radicle. Barley and wheat grains germinated in water of 2 mm. depth contained more catalase than those germinated under 4 mm. of water. They refused to germinate in water deeper than 6 mm., and showed very slight or no increase in catalase activity. According to THORNER (18) they

do not retain vitality very long under these conditions. At this depth normal development of chlorophyll is interfered with. The plumules remain pale green, and in boiled water developed no chlorophyll at all. The catalase activity does not increase at greater depths, but actually decreases, even though growth may continue in the medium devoid of oxygen. In experiment no. 7, using ten seedlings, the catalase decreased from 53.9 cc. oxygen equivalent to 27.2 cc. equivalent in 144 hours. The average growth of plumules here was 12 mm.

As to normal respiration, the aerobic seedlings with much catalase, compared with anaerobic seedlings, took in about twice the volume of oxygen in 20 hours. The respiratory ratio was much greater than unity at the beginning of germination. This fact was more conspicuous for the anaerobic seedlings. These two kinds of seedlings, aerobic and anaerobic, gave off carbon dioxide in the ratio of 1:0.78 in anaerobic respiration; and the amount of carbon dioxide given off by the anaerobic seedlings in both kinds of respiration was nearly the same.

From the data as a whole, the writer concludes that there is a close relation between the amount of catalase activity in the seedling and the amount of free oxygen in the medium in which it germinated, and that catalase seems to have a close positive connection with the normal respiration, even if it seems to have some retarding effects, directly or indirectly, on the fermenting process estimated by the amount of carbon dioxide given off.

Summary

1. The amount of catalase in the dry seeds of rice is much less than that of wheat, barley, oats, and rye, being in fact about one-tenth as great.
2. Rice germinating aerobically contains much catalase, about seven-tenths as much as germinating wheat, barley, and oats.
3. The catalase does not increase in the course of anaerobic germination, but it does slowly in the course of germination in a medium which contains a reduced amount of oxygen. Hence the ratio of increase of catalase activity is a function of the free oxygen in the medium.

4. The free oxygen affects directly or indirectly the development of plumule and radicle, and also of the chlorophyll in it.

5. The catalase once increased by aerobic conditions decreases during anaerobiosis, while growth of the plant continues.

6. Aerobically grown seedlings with high catalase use much more oxygen than do anaerobically grown seedlings with low catalase activity. The anaerobic seedlings, however, give off a comparatively large amount of carbon dioxide during respiration, whether under normal aerobic or under anaerobic conditions.

The writer takes pleasure in expressing here his cordial thanks to Dr. CHARLES A. SHULL, with whom this work was undertaken, and to Dr. SCOTT V. EATON for their valuable suggestions and criticism given throughout the progress of the work.

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DIFFERENTIATION OF SPORANGIA IN MARSILIA QUADRIFOLIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 332

CORNELIA C. MARSHALL

(WITH NINE FIGURES)

Most of the stages in the development of the megasporangium of *Marsilia quadrifolia* have been described. It is the purpose of this paper to reexamine the work done by others, to add a few stages, but particularly to show where the differentiation of the sporangia occurs.

The material for this investigation was collected and kindly sent by Miss ETHEL THOMAS from Charleston, Illinois. Some of the material was fixed in chromoacetic acid with one cubic centimeter of osmic for every hundred cubic centimeters of the solution; and some was fixed in corrosive sublimate heated to 85°. The sections were cut 8-10 μ thick and stained with Haidenhains' iron-alum haematoxylin.

Historical

JOHNSON (2) determined that in each sorus the megasporangia are derived from a single megasporangial cell, and the microsporangia from two microsporangial cells formed in the basicopic marginal cell of each soral segment. These megasporangial and microsporangial cells are sister cells. The microsporangial cells increase in number by radial growth and division. The megasporangium mother cells are pushed out by the growth of the plerome into the soral cavity far beyond the microsporangium mother cells. They swell laterally to several times their former size, and in so doing push the microsporangial cells around to a position nearly at right angles to their former one.

The megasporangium mother cell finally divides by three inclined walls to form the tetrahedral apical cell of the megasporangium. This apical cell cuts off two more segments on each of the three sides, which form a stalk and basal wall cells of the sporangium.

Then a pericline is formed near the outer end of the apical cell, cutting off the archesporium and completing the sporangium wall. While the microsporangial cells are being pushed aside, each has divided by anticlines approximately parallel to the segment wall, first to two, then to four. These come to lie parallel with the segments of the apical cell of the megasporangium. Of the four cells formed from each of the microsporangial cells, the lower three form sterile tissue of the placenta, while only the upper one, next to the megasporangium, actually forms microsporangia. Each of these upper cells divides by walls transverse to the axis of the sorus to form four cells of each side of each megasporangium. Then each of these four cells swells out to the placenta and divides into a basal cell and an outer cell, in which outer cell is formed later the tetrahedral apical cell giving rise to the stalk, wall, and archesporium.

According to CAMPBELL (1), the checking of the apical growth in the megasporangium results in a very short stalk; in the microsporangium the stalk is longer. The archesporial cells by a series of divisions give rise to a well developed tapetum of two and often three layers. At the same time sixteen sporogenous cells are formed. CAMPBELL also states that soon after the first divisions of the central cells are completed the cell walls of the tapetum begin to dissolve, but for a time the sporogenous cells remain together. Next they become isolated and round off for their final divisions into spores. In stained sections the nucleated protoplasm of the tapetal cells is very evident after the walls have disappeared. He states: "At this time the difference in the two sporangia becomes manifest. Those in the lower part of the sorus, that is the oldest ones, form megasporangia, the upper ones microsporangia."

The young spores of each tetrad show a marked tendency to hang together by strong protoplasmic strands, as was first shown by STRASBURGER (8). SEATTUCK (6) states: "These strands are the first recognizable morphological feature by which one can determine that a sporangium is to form a megaspore rather than microspores." These strands persist until the megaspore is quite mature, and in many instances can be seen on the papilla of the germinating megaspore, which still subtends the three members of the tetrad.

In the microsporangium every one of the sixteen mother cells produces four functioning microspores, sixty-four in all; while in the megasporangium only one spore out of sixty-four, all of which seem to be identical in every particular when the tetrads are first formed, produces a megaspore. A closer examination reveals the fact that this one megaspore does not gain ascendancy without a struggle. In fact, many instances were found in which there was rivalry between two enlarging potential megaspores. Yet in the end, one centrally placed spore always gains the ascendancy and the others disintegrate.

Investigation

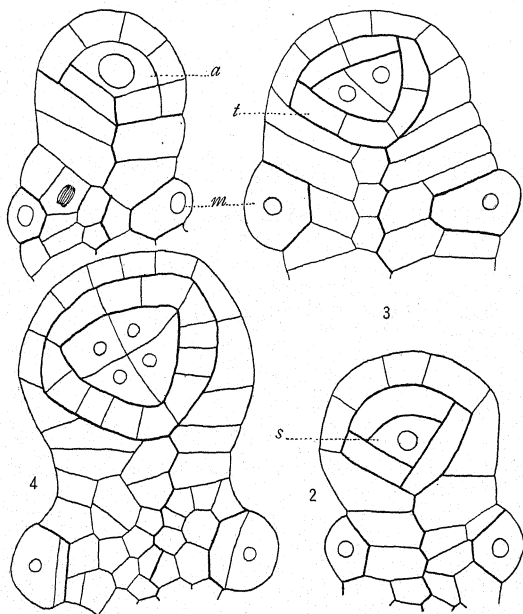
The early development of the megasporangium proceeds in the usual leptosporangiate manner, the central tetrahedral archesporial cell cutting off, by periclinal walls, the primary tapetal cells, which are easily distinguished from the outer cells by their more granular appearance. The outer cells divide by transverse divisions (figs. 1, 2).

The single sporogenous cell divides soon after the first tapetal cells have been cut off. The first division is usually transverse, but it may be more or less inclined or even vertical (fig. 3). Then, at right angles to this wall, two successive divisions occur, the first resulting in the quadrant stage, the second in the octant stage (fig. 4). These eight cells divide again, giving rise to the sixteen spore mother cells (fig. 5).

During the division of the sporogenous cells the tapetal cells divide by both anticlinal and periclinal walls, forming a tapetum of two or three layers of cells, a more extensive tissue than is found in the Polypodiaceae. The epidermal cells also divide by a number of transverse divisions. These outer cells are nearly uniform, and show not even the beginning of any differentiation into stomium or annulus; consequently, there is nothing to indicate the ancestry of *Marsilia*, so far as this might be determined by annulus characters.

The microsporangia, which appeared as sister cells of the megasporangium, are at this time found below the megasporangium. While the megasporangia were developing sporogenous tissue, the apical cell of the microsporangia was cutting off segments to form

an elongated stalk. When the megasporangium reaches the sixteen-spore mother cell stage, the microsporangia cease to add any more segments to the stalk; a transverse wall through the apical cell of



FIGS. 1-4.—Fig. 1, megasporangium with single archesporial cell (*a*); *m*, microsporangia; fig. 2, primary sporogenous cell (*s*); fig. 3, quadrant stage; tapetum (*t*); fig. 4, octant stage.

the microsporangium cuts off a cap cell, and a central tetrahedral archesporial cell is established.

The megaspore mother cells are so elongated that in longitudinal or transverse sections the group of sixteen cells is nearly twice as

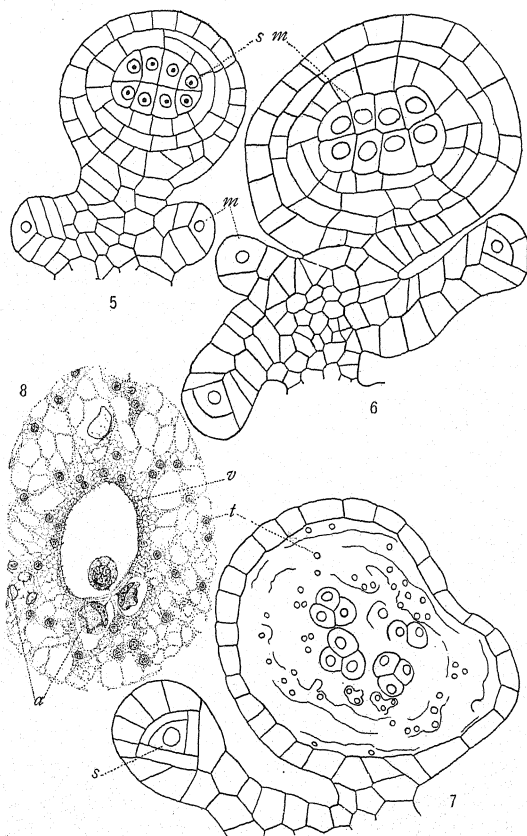
long as broad. They are easily distinguished from the tapetum and the epidermal cells by their denser finely granular cytoplasm, and by the fact that the nucleus soon becomes very large. The nuclei of the sporogenous cells are larger than those of the tapetum, and the nuclei of the tapetum are larger than those of the epidermis.

A number of mitotic figures in the nuclei of the sporangia showed the chromosomes in synapsis, diakinesis, anaphase, metaphase, and telophase. An examination of the nucleus in synapsis shows a clear space at one side. The chromatin is in a somewhat contracted mass at the other side, and the outline of the individual chromosomes is just discernible. The nucleolus may be closely associated with the chromatin mass or it may be at some distance. The nuclear membrane is quite distinct.

A pair of large chromosomes is observed in an early diakinesis stage. The sixteen pairs of chromosomes are attached to each other by threads. A little later the pairs become more clearly differentiated and the single large pair stands out more distinctly. The continued appearance of this large pair of chromosomes suggests that they may be sex chromosomes.

The x or haploid generation of *Marsilia* is very short, since there are only a few divisions from the megaspore up to the egg ready for fertilization; and there are also only a few divisions from the microspores to the production of sperms. At fertilization a monoecious $2x$ generation is established, bearing both megasporangia and microsporangia in the same sporocarp, so that the segregation of the sexes must have taken place in some somatic mitosis at an early stage in the development of sporangia. Since the megasporangia and microsporangia are derived from sister cells, sex differentiation should occur in the formation of these cells. The fact that in the megasporangium the spore mother cell showed a large pair of chromosomes may indicate that segregation has occurred previously. As soon as more material is available, the chromosomes of the microspore mother cell will be examined and compared with the megaspore mother cells and with the somatic cell from which they are derived. Should these three differ in chromatin content, this will be conclusive evidence that the segregation of sexes occurred as stated.

With the reduction divisions in the sixteen megaspore mother



FIGS. 5-8.—Fig. 5, sixteen megaspore mother cells; microsporangia (*m*); fig. 6, same slightly older; fig. 7, megasporangium with tetrads; tapetal plasmodium (*t*); microsporangium with archesporial cell (*s*); fig. 8, functioning megaspore; vacuole (*v*); tapetal nuclei (*t*); abortive spores (*a*).

cells, sixty-four spores are formed. Each tetrad of spores hangs together by strands, and all are surrounded by protoplasm and nuclei of the tapetum, cell walls of which have been dissolved (fig. 7). The microsporangium at this time shows that the first tapetal cells have been cut off and the epidermal cells have divided transversely.

Only one of the sixty-four megaspores ever reaches maturity. The centrally placed spore which gains ascendancy is surrounded by a mass of vacuolated cytoplasm, in which the disintegrating tapetal nuclei as well as the abortive spores may be found. This mass is gradually assimilated by the maturing megaspore (fig. 8). Two megasporangia are sometimes found developing simultaneously in a single sorus (fig. 9). At this time the microsporangia have reached the sixteen-spore mother cell stage. Very soon each spore mother cell forms a tetrad of microspores, the tapetal walls are dissolved, and the nuclei and protoplasm of these cells surround the microspores.

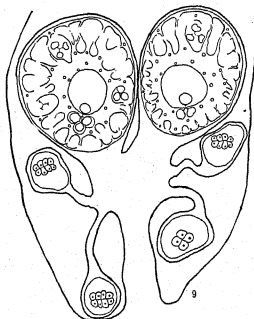


FIG. 9.—Diagrammatic section of two megasporangia; microspore mother cells.

Finally, the megasporangia and microsporangia reach their full development at the same time; in the former only one megaspore maturing, while in the latter sixty-four microspores mature. A single layer of epidermal cells is all that remains of the sporangia, and this layer, even in the latest stages, shows no indication of an annulus or any special structure for dehiscence.

Comparison with other heterosporous forms

It is interesting to compare *Marsilia* with the other heterosporous Pteridophytes, and to note where the differentiation of sporangia occurs in each.

In *Selaginella* (4) the sporangia develop nearly alike up to the

spore mother cell stage, but, even before sporogenous divisions cease, a slight elongation distinguishes the microsporangia, while the megasporangia can be identified by a more nearly spherical shape. In the microsporangia all the mother cells function and numerous microspores are formed, while in most species in the megasporangia all but one of the spore mother cells abort and supply nutrition to the four functioning megaspores.

In *Isoetes* (7) the sporangia develop alike until they reach the 15,000-20,000 cell stage. Then, in those sporangia which are to form microspores, some of the sporogenous cells are diverted and form trabeculae which extend across the sporangium, while the remainder function in forming the microspores, varying in number from 150,000 to 300,000. In those which are to form megaspores, some of the sporogenous tissue forms trabeculae, which, however, are more massive; the remainder function in the production of the 150-300 megaspores.

In the sporocarp of *Azolla* (5) the megasporangium develops first, followed soon by the microsporangium, after which comes a period of simultaneous growth. Observation shows that if the apical sporangium gains the ascendancy, it becomes a megasporangium, and lateral sporangia abort. Within the functioning megasporangium 31 of the 32 megaspores abort, leaving one functioning megaspore. On the other hand, if the apical sporangium aborts the lateral sporangia become microsporangia, and their 30 spores become microspores.

In *Salvinia* (9) the microsporangia and megasporangia develop in different sporocarps. Several megasporangia are produced in a single sporocarp, each sporangium producing one megaspore. In the microsporocarp the numerous sporangia mature all their spores.

In *Pilularia* (3) the first sporangia formed, near the base of the sorus, produce megaspores, while the upper and younger ones produce microspores. As in *Marsilia*, the sporangia are all produced in the same sporocarp. While this does not show exactly where differentiation of sex in the water ferns occurs, it at least indicates, within rather narrow limits, where any structural differentiation is to be sought.

Summary

1. The microsporangia and megasporangia of *Marsilia* are derived from sister cells.

2. The stalk of the microsporangium is very much longer than that of the megasporangium.

3. In the spore mother cell stage the microsporangia are elongated and oval, while the megasporangia are somewhat flattened.

4. The sporangia differ in their time of development. In the megasporangium there is a gradual continued development of the sporogenous tissue; in the microsporangia there is delay in the development of the sporogenous tissue, while the apical cell cuts off segments to form an elongated stalk.

5. The megaspore mother cell in diakinesis shows a pair of large chromosomes which may be sex chromosomes.

6. Since *Marsilia* is a monoecious plant, sex differentiation must have occurred in some somatic mitosis.

7. When the single functioning megaspore has gained ascendancy, the sporogenous tissue of the microsporangia resumes growth and develops very rapidly.

8. The protoplasmic strands between the tetrads of megaspores are much stronger and more persistent than in the microspores.

9. The functioning megaspore is surrounded by a mass of vacuolated cytoplasm, in which the tapetal nuclei as well as the aborted spores may be found.

10. In each of the six genera of heterosporous Pteridophytes the difference in the sporangia becomes visible at a different stage. If the chromosomes were examined it would probably be found that the differentiation of sex had occurred in an earlier somatic cell.

Grateful acknowledgment is made to Professor CHARLES J. CHAMBERLAIN for criticism and suggestions.

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SYMBIOSIS IN A DECIDUOUS FOREST. II

W. B. McDougall

(WITH ONE FIGURE)

The first paper of this series¹ was devoted to an ecological description of University Woods, in Champaign County, Illinois, and to a general discussion of social disjunctive symbiosis as exhibited therein. It is the purpose of the present installment to discuss briefly some instances of nutritive disjunctive symbiosis and their effect on the vegetation in this same woods. It should be understood that this is not intended as an exhaustive treatment of the subject, which would require many years of observation and study. The purpose is rather to present the relatively few examples that have been studied during the past few years, not only as a contribution to our knowledge of symbiosis, but also to illustrate what kinds of phenomena should be included under nutritive disjunctive symbiosis.

Antagonistic nutritive disjunctive symbiosis

The phenomena that are classed under this type of symbiosis are those in which two or more unlike organisms are living together (not in actual contact, at least not all of the time) and one or more (but not all) kinds of organisms deriving food from the others. All ordinary cases of the interrelations of herbivorous animals and plants, therefore, belong in this category.

Before the University of Illinois obtained possession of the University Woods some seven years ago, it was much grazed by cattle. This had the effect of practically eliminating some species of herbaceous plants and of greatly reducing the numbers of others, while reproduction of the trees was for the time being almost completely stopped. The effects of this overgrazing are still very evident. Since the woods was acquired by the University there has been no grazing, and reproduction of the trees is now progressing satisfactorily. Certain herbaceous plants which were unpalatable to the

¹ Bot. Gaz. 73:200-212. 1922.

cattle survived the period of grazing in such numbers that they were able to take advantage of the weakened condition of other species and spread rapidly. A notable example of this is *Laportea canadensis*. Fig. 1 shows the present distribution of this plant. The species is undoubtedly much more abundant than it would be if the woods had never been grazed. Another plant which has likewise become very abundant, especially in those parts of the woods not occupied by the nettle, is *Eupatorium urticaefolium*.

Other herbivorous mammals have not been important in this woods. Rabbits feed upon various kinds of plants without doing much damage. Squirrels, mice, and perhaps a few other rodents feed upon nuts and other fruits, but they have never been abundant enough to become very important symbionts.

Instances of antagonistic, nutritive, disjunctive symbiosis between insects and plants are numerous. The majority of these, however, do not have any very marked effect on the life of the plants, although occasionally some of them do. Perhaps the most effective case observed is that of some unidentified lepidopterous larvae on the wood nettle.* These feed upon the leaves, and are often so abundant during late summer and early autumn that it is scarcely possible to find a single intact leaf. Such a condition is rather serious for the plant, of course, although it is unlikely that the plants are ever actually killed by the larvae. Several kinds of lepidopterous larvae were also found feeding on the leaves of *Benzoin melissaefolium*.

Lace bugs were found rather commonly on the under side of the leaves of *Asimina triloba*, where they feed on the plant juices. The tarnished plant bug, which feeds entirely on plant juices, was found frequently on several species of *Aster* and *Solidago*. Aphids, often attended by ants, were found on various kinds of plants, especially members of the composite family. These are only a few of the cases of symbiosis between insects and plants that might be found in such a forest, but they all represent interrelations entirely comparable with that of the well known leaf-cutting ants and the fungi which they cultivate, and therefore should be classified under the same type of symbiosis, namely antagonistic, nutritive, disjunctive.

* The observations on symbiosis between insects and plants recorded here were made by Mr. CURTIS BENTON.

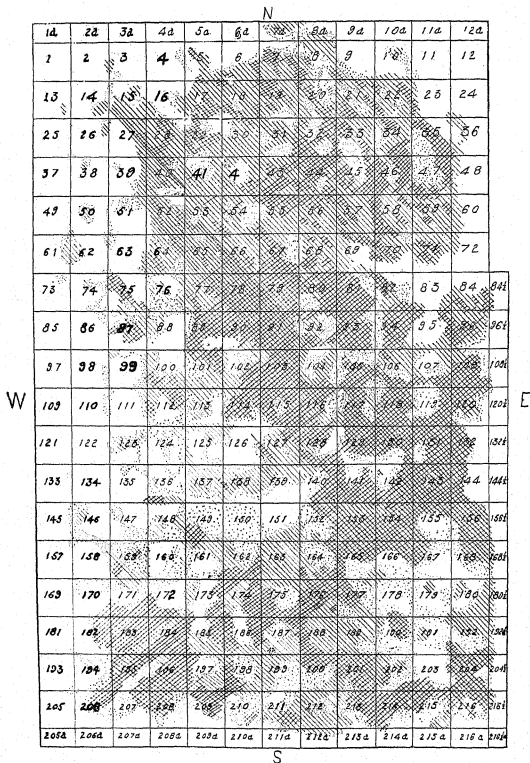


FIG. 1.—Present distribution of *Laportea canadensis* in University Woods: double diagonals, dense growth; diagonals, medium density; dots, scattered plants; chart prepared by P. E. GROVE.

The most conspicuous example of this type of symbiosis in the University Woods, and the one that produces the greatest effect on the vegetation, is that between the domestic fowls and the plants. There are two farmhouses across the road from the woods on the west. One of these is opposite quadrat no. 73 (fig. 1) and the other opposite quadrat no. 157. A flock of chickens is maintained at each of these farms. These enter the woods daily and occasionally go as far as half way across the woods. Usually they do not go so far, however, and the effects of their presence is most noticeable in the first two or three rows of quadrats. The chickens feed upon the plants directly to a certain extent, and they also do considerable damage by scratching the soil in search of insects and other food. The greater part of the food obtained by the chickens is probably not obtained directly from the plants, but from the point of view of the chickens, their relationship with the plant community concerns their food supply, and therefore the symbiotic relation is a nutritive one. On the other hand, the plants do not get any food from the chickens and probably are not benefited in any other way by their presence. Therefore the relationship is an antagonistic one.

The method used for estimating the effect of the presence of the chickens on the vegetation was as follows. It will be noticed from fig. 1 that the woods is divided into 216 full quadrats, with half quadrats along each end and part way along the east side. Each of the full quadrats is 100 feet square, and each half quadrat is 50 by 100 feet. At one time the corners of all of these quadrats were marked by stakes, but these have mostly disappeared. It was decided to get a comparison between the west and east sides of the woods by counting the total number of plants of all species in a series of meter quadrats along each side 100 feet from the fence. It was further decided to locate these meter quadrats at every 100 feet. Starting at the southwest corner of the woods, therefore, the first quadrat was located by pacing 33 paces east and 33 paces north to the middle of the north and south boundary line between quadrats 205 and 206. Then using a compass to keep on the line, the next quadrat was located by walking 33 paces due north to the middle of the line between quadrats 193 and 149, and so on until the series had been completed. On the east side of the woods the same method was used, but in the southern two-thirds the meter quadrats

fell in the middle of the large quadrats of the first row of full quadrats, instead of on the line between the first and second rows, as on the west side and in the northern third of the east side, this variation being due to the jog in the fence on this side of the woods.

A folding wooden frame one meter square which was easily carried about was used, so that when the position of a quadrat had been located it was only necessary to drop the frame and proceed at once to count the plants within it.

TABLE I

WEST SIDE		EAST SIDE	
Quadrat	No. of plants	Quadrat	No. of plants
A.....	25	a.....	70
B.....	32	b.....	41
C.....	12	c.....	21
D.....	15	d.....	49
E.....	9	e.....	54
F.....	3	f.....	59
G.....	34	g.....	56
H.....	6	h.....	54
I.....	19	i.....	62
J.....	80	j.....	30
K.....	33	k.....	90
L.....	28	l.....	71
M.....	21	m.....	41
N.....	12	n.....	42
O.....	5	o.....	45
P.....	16	p.....	59
Q.....	33	q.....	44
R.....	25	r.....	45
Average.....	22.7	51.8

The plants in the eighteen quadrats thus located on the west side of the woods were counted on June 5, 1923, and those on the east side were counted two days later, on June 7. A second counting was made in the quadrats on the west side on August 27 and on the east side on August 30. In order to avoid confusing the meter quadrats with the one hundred feet quadrats, the small ones were designated by letters instead of numerals. Beginning at the south end of the woods they were lettered from *A* to *R* on the west side of the woods, and from *a* to *r* on the east side. The results of the first counts made, those of June 5 and June 7, are shown in table I.

It is seen from table I that the average number of plants per meter quadrat on the west side of the woods where the chickens work is less than half that on the east side. The total number of species represented in the 18 quadrats on the west side of the woods was 31, while in the 18 quadrats on the east side there were only 28 species represented. This difference is to be explained by the fact that the activities of the chickens in destroying the native plants made possible the entrance of a number of ruderal species which were not represented on the east side at all. Nineteen species were common to the quadrats on the two sides of the woods.

The area of greatest activity of the chickens, however, is confined to about 500 feet of north and south extent for each flock. Therefore, if we disregard quadrats *A*, *B*, *C*, and *D* at the south, *J* and *K* in the middle, and *Q* and *R* at the north, we have left the ten quadrats that show the effect of the chickens to the greatest extent. The ten quadrats on the east that can best be compared with ten on the west are those at the south end, *i* to *r* inclusive, since they are most like the west side of the woods as regards dominant species; the south end of the east side and practically all of west side being in the maple consociation, while the north end of the east side is in the elm consociation.

Comparing these selected quadrats, we find in the ten on the west side a total of 153 plants, or an average of 15.3 per quadrat, and in the ten on the east side a total of 496 plants, or 49.6 per quadrat. The total number of species represented in the ten quadrats on the west side was 21, while the total number on the east was 18. Twelve species were common to the two sets of quadrats.

The results of the counts made in August are shown in table II. The totals are lower than in June, owing to the fact that the vernal plants had for the most part disappeared and their places had been taken by the larger plants of the aestival season, but in general the results are similar to those obtained in June.

The total number of species represented in the 18 quadrats on the west side in August was 27 and on the east side 21, while eleven species were common to the two sets of quadrats.

By using only the ten selected quadrats on each side of the woods, as was done for the June counts, we find the average number

of plants per quadrat on the west side to be 12.2 and on the east side 28.4. The total number of species represented in the ten quadrats on the west side was 16 and in those on the east 11, while only four species were common to the two sets of quadrats.

The conclusion may be drawn from these data that the presence of the chickens in the woods tends to reduce greatly the number of individual plants but to increase the number of species. This

TABLE II

WEST SIDE		EAST SIDE	
Quadrat	No. of plants	Quadrat	No. of plants
A.....	39	a.....	21
B.....	35	b.....	33
C.....	20	c.....	39
D.....	14	d.....	14
E.....	16	e.....	26
F.....	3	f.....	25
G.....	11	g.....	26
H.....	30	h.....	35
I.....	14	i.....	25
J.....	13	j.....	40
K.....	26	k.....	32
L.....	14	l.....	34
M.....	10	m.....	31
N.....	10	n.....	27
O.....	5	o.....	18
P.....	9	p.....	27
Q.....	16	q.....	26
R.....	19	r.....	24
Average.....	16.8	28

latter effect, as just stated, is due to the introduction of weedy plants which are out of place in the forest and could not possibly compete with the forest plants under normal conditions. The presence of the chickens is therefore distinctly detrimental to the forest vegetation; it is a clear case of antagonistic, nutritive, disjunctive symbiosis.

Reciprocal nutritive disjunctive symbiosis

In this type of disjunctive symbiosis one or more, but not necessarily all, of the organisms concerned get food from one or more of the others, but those that lose food and get none in return are never-

theless benefited by the relationship in some other way, so that the symbiosis is reciprocal so far as actual benefit derived from it is concerned. The outstanding examples are those concerned with pollination by means of animals, especially insects, and with dissemination through the aid of animals, especially birds and ants. Since my observations on this type of symbiosis have not brought out any new facts, a brief statement of the extent to which it occurs in this woods will suffice.

Of the 132 species of herbaceous plants occurring in the woods, 101, or approximately 76 per cent, are pollinated by insects, while the insects are getting food from the flowers. Of the 31 species of trees, only 9, or approximately 29 per cent, are insect pollinated; but all of the twelve species of shrubs and all of the six species of lianas have this symbiotic relation with insects. It will be seen that the small amount of space I am giving to this type of symbiosis is not in proportion to its importance to the plants.

Dissemination through the aid of animals is not so widespread as is pollination, but it is by no means unimportant. There are, of course, a number of plants with burlike fruits which are disseminated by animals, but those cases do not represent the type of symbiosis now under consideration, because there is no food relation. Birds are by far the most important agents which bring about dissemination through their activities in connection with their food supply. Thirteen herbaceous plants, or about 10 per cent of the total number, are disseminated to a certain extent by birds. Six species of trees, or 19 per cent of the total number, and all of the twelve species of shrubs and six species of lianas exhibit this type of symbiosis.

Dissemination by insects is not so common, but ants feed upon the large crests of the seeds of *Sanguinaria canadensis* and upon the fleshy raphes of the seeds of *Asarum canadense*, and frequently carry the seeds from place to place, thus aiding in scattering them. They probably also have a similar symbiotic relation with *Floerkea proserpinacoides*, feeding upon the rough, fleshy outer portions of the akenes, although further observations are needed upon this point.

BRIEFER ARTICLES

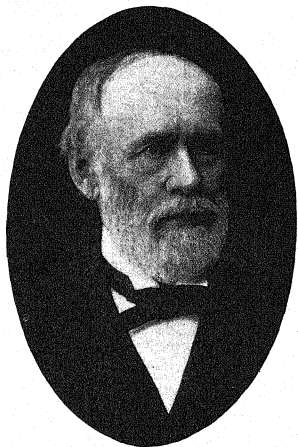
WILLIAM JAMES BEAL

(WITH PORTRAIT)

With the death of Dr. BEAL, May 12, 1924, there passed away the last of those leaders of botany who, in the latter half of last century, were changing the course of botanical thought from the idea of systematic botany as the end and aim of all botany to the study of all kinds of plants from all standpoints.

Dr. BEAL was born at Adrian, Michigan, March 11, 1833, of Quaker stock. His parents were pioneers in Michigan, which was then, for the greater part, still wilderness. Taking advantage of the meager opportunities for schooling, he made his way through the district school and seminary and entered the University of Michigan, from which he graduated in 1859. Most of his education was obtained at the cost of hard work, for it was necessary for him to earn his way through the academy by the then usual

method of teaching school during the winter. After graduation, he became teacher of Natural Science at the Friends' Academy, and later at Howland Institute at Union Springs, New York. This position he held, except for absences for the purpose of further study, from 1859 to 1868. During vacations and other periods he found opportunity to study at Harvard, coming under the influence of LOUIS AGASSIZ and ASA GRAY, the former probably making the greater impression upon him, as shown in



his teaching methods later. In 1865 he received the degree of B.S. from Harvard.

In 1868 the original University of Chicago called him to be Professor of Botany, a position which he held for two years. Upon the call of Professor PRENTISS from the Michigan Agricultural College to Cornell University, Dr. BEAL was made lecturer in botany for part of the year 1870 at Michigan. In 1871 he became a full member of the faculty as Professor of Botany and Horticulture. In 1882 the Department of Horticulture was established, and he then became Professor of Botany and Forestry. When a separate Department of Forestry was formed in 1903, Dr. BEAL was relieved of the forestry work and became Professor of Botany alone. In 1910, at the completion of his fortieth year of connection with the Michigan Agricultural College, he retired as Professor Emeritus and went to Amherst, Massachusetts, where he resided at the home of his daughter, Mrs. RAY STANNARD BAKER, until his death.

Dr. BEAL was always a man of abstemious habits, to which, coupled with his habitual practice of exercising, he ascribed his freedom from illness up to about the very end of his life. When far past his eightieth year he ran a few hundred feet every day or sawed a set number of sticks of wood. He outlived most of his University of Michigan classmates, and at the time of his death was the oldest graduate of that University, and the oldest male resident of Amherst, Massachusetts.

Interested in the diffusion of scientific knowledge, Dr. BEAL associated himself with various scientific societies. He was one of the organizers and the first president of the Society for the Promotion of Agricultural Science, and charter member and first president of the Michigan Academy of Science. He was Director of the State Forestry Commission from 1888 to 1892, Fellow of the American Association for the Advancement of Science (chairman of the Section of Biology in 1895), Secretary of the American Pomological Society from 1881 to 1885, President of the Michigan State Teachers' Association, member of the Botanical Society of America, and of a number of other societies. In all these he took an active part until about the time of his retirement in 1910.

As a writer, Dr. BEAL's most extensive botanical contribution was the two volume work *The grasses of North America*, published in 1887 and 1896. He published a small booklet in 1879 entitled *The new botany*, and one in 1898 on *Seed dispersal*. His greatest literary activity, however, was in articles for botanical periodicals and for horticultural and agricultural papers. Particularly numerous were the papers of the last class, and it was by means of these that Dr. BEAL became so widely known among the citizens of Michigan and adjoining states.

Probably it was as a teacher that Dr. BEAL had his widest influence. He came to the teaching profession with a breadth of experience and training unusual in those days. As a boy and young man he knew the practical side of agriculture and horticulture from personal experience. His university course was of the classical-literary type, the only kind available then, apart from the professional courses leading to law, medicine, or the ministry. At Harvard, under GRAY and AGASSIZ, he studied botany and zoology, subjects which had always been of great interest to him. Under AGASSIZ he first encountered the laboratory as a method of instruction in biological science, for GRAY's undergraduate courses at that time were still given only as recitations from a text-book, without requiring the students to see the plants mentioned. This made a lasting impression on Dr. BEAL, so that when he came to Michigan Agricultural College he was not satisfied to teach these subjects by the text-book and recitation method. It was not long before he managed to set aside a small space for a botanical laboratory, and planned for a laboratory building. An interesting fact is that GOODALE of Harvard, BESSEY of Iowa, BURRILL in Illinois, and BEAL in Michigan, independently of one another, all adopted the laboratory idea for teaching undergraduate botany at about the same time, in the early seventies. In 1879 Dr. BEAL succeeded in obtaining an appropriation to build a botanical laboratory, a two-story wooden building, one of the first buildings in this country to be devoted to botany alone. This was occupied in the spring of 1880, and was destroyed by fire in 1890, but the general herbarium, apparatus, and books were saved.

In addition to his manifold duties as Professor of Botany and Horticulture, with no assistance in teaching, Dr. BEAL started a botanical garden in 1877. This is, therefore, one of the older botanical gardens of the country, and at the close of Dr. BEAL's service in 1910 it contained over 2000 species.

In 1879 Dr. BEAL began his well known experiment with buried seeds. These seeds were mixed with soil and placed in uncorked bottles, buried mouth downward in the ground at a depth of 16-24 inches. Every five years one bottle was dug up and the seeds germinated, and since Dr. BEAL's retirement this has been continued. At the fortieth year eleven kinds of seeds still showed germination out of the twenty kinds placed in the bottle originally.

Dr. BEAL held ideas that were far in advance of the time. In the mid-seventies he called attention repeatedly to the folly of the destruction of the forests, and advocated the treatment of forests more in line with what is now considered to be sane forest conservation. He was ridiculed greatly for his fears for the "inexhaustible forests of Michigan," but lived to see

the day when the state began the replanting of cut-over land. As a recognition of his interest in forestry, he was made Director of the State Forestry Commission from 1888 to 1892.

The final botanical publications of Dr. BEAL were two bulletins on *Seeds of Michigan weeds* and *Michigan weeds*. For over thirty years he had studied the problem of agricultural seeds and their impurities and poor quality, contending for cleaner, better seed. In 1877 he began testing seeds for purity and viability long before the first seed laboratory was established in this country. In 1910 his bulletin on *Seeds of Michigan weeds* showed that he had not yet lost interest in this important subject. It would be impossible to list the thousand or more articles he contributed to various agricultural and horticultural journals.

After his retirement, Dr. BEAL moved to Amherst, Massachusetts, giving up all botanical work. His large collection of botanical separates he gave to the college, and spent a year or two writing a history of Michigan Agricultural College. Although ceasing botanical work, he did not lose his interest in plants, and almost up to the time of his death he worked in his garden, set out shrubbery, and dispensed information of practical value on these subjects to his friends and neighbors.—ERNST A. BESSEY.

EFFECT OF LIGHT ON AVAILABILITY OF IRON TO WHEAT PLANTS IN WATER CULTURES

(WITH ONE FIGURE)

How different climatic complexes may markedly affect the growth efficiencies of aqueous culture media, can be very nicely demonstrated in experiments with nutrient solutions devoid of iron. It appears that deficiency or absence of this element in the media has a much more deleterious effect on plants growing in bright sunlight than it has on those growing under conditions more or less shaded.

So markedly does light affect the availability of or the requirement of the plant for iron, that demonstrations for the classroom on the effect of this element on the greening of plants often fail to give the desired results because of certain photogenic conditions. Fig. 1 shows some of the effects of two different light intensities on the growth of wheat in nutrient solutions devoid of iron; the chief points of difference in the plants being that those exposed to bright sunlight were much more etiolated, showed marked abbreviation in length of leaves and stalks, and possessed more incipient tillers than did those plants exposed to less sunlight.

The features of the plants exposed to the high light intensity apparently are the result of conditions that produced a higher rate of operation of certain growth processes than prevailed with the plants grown under a lower light intensity. Due to these differences in rates of processes, the requirement of the plant for iron may vary. It appears that the need for this material is much greater per unit of growth of plant exposed to high light intensities than to low ones.

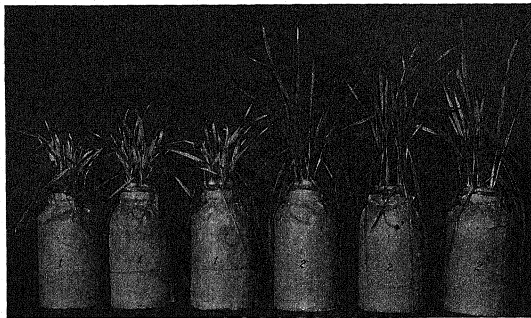


FIG. 1.—1, wheat plants grown in solutions devoid of iron and exposed to bright sunlight; 2, wheat plants grown in similar solution devoid of iron, but exposed to less light.

The marked etiolation of the plants exposed to bright sunlight has been shown to be related to certain effects of light on chlorophyll. Apparently both the amount of growth, and the stage of growth that accrues from the operation of specific rates of various processes, can be affected by this agency. Although the plants exposed to bright sunlight were much smaller than those not so exposed, the former were more matured than the latter; that is, exposure of the plants to conditions of relatively high light intensity without iron in the media resulted in more matured but stunted plants, whereas with iron in the media under the same light conditions the plants would have been larger. Thus conditions, which in the presence of iron in the culture media are decidedly beneficial to growth, become conditions relatively harmful in the absence of iron. These results, therefore, indicate that there can be no fixed minimum amount of

iron for a unit yield of product, and as this principle applies to iron, so may it possibly apply to all other elements required for the growth of plants. The amount of material needed by plants for a unit yield of product would be determined by rates of other processes that are part of the complex-growth.

While many of the synthetic products of plant origin have a definite chemical composition and structure, and therefore involve stoichiometric relations of their component parts, nevertheless, as the composition of plants is, in a sense, a mixture of various chemical compounds, it follows that any change in the proportion of the components of this mixture must also affect the amount of any given element required per unit growth of plant.—W. F. GERICKE, *Laboratory of Plant Nutrition, University of California*.

CURRENT LITERATURE

BOOK REVIEWS

Textbooks of general botany

HOLMAN and ROBBINS,¹ of the University of California, have published a textbook of general botany, which contains the material presented in two courses, one for agricultural students at the Branch of the Agricultural College at Davis, and one for both agricultural and liberal arts students at Berkeley. Part I begins with the cell, followed by chapters on the plant as a whole, stem, root, leaf, flower, fruit, seed, and seed germination. Part II presents the four great groups of the plant kingdom, closing with a chapter on evolution and heredity.

Every teacher with any initiative has his own method of presenting the facts of his subject, and in consequence textbooks of botany are multiplying rapidly. It is very interesting to discover in this way the methods of different teachers. The present volume is written in clear style, including much detail, and is amply illustrated. Among the illustrations used there are selections from a wide range of authors. Naturally, the presentation of the material has been adjusted to the needs of the particular groups of students involved, and has been tried with the student groups before publication.

A group of botanists at the University of Wisconsin has co-operated in the publication of a textbook of general botany.² It is based upon the conviction that botany should be presented as a unit, and is an outgrowth of the experience of the writers in the teaching of elementary botany. Especially have they emphasized the fact that structure and function should be intimately associated. The six authors are not differentiated in the presentation, but it is a synthesis of their training and teaching experience. The result is certainly interesting and very suggestive, as is also the sequence of presentation.

The first twelve chapters deal with the general structures and functions of plants, beginning with the cell, and proceeding through roots, stems, buds, and leaves to water relations, food manufacture, plastids and pigments, food utilization, stimulus and response, and embryogeny. The following eighteen chapters deal with the great groups, beginning with algae and closing with seeds and fruits. A chapter between mosses and ferns is devoted to an account of reduction of the number of chromosomes. The closing chapters deal with inheritance and variation, evolution, geographic distribution, and economic significance of

¹ HOLMAN, R. M., and ROBBINS, W. W., *A Textbook of general botany*. 8vo. pp. vii+590. figs. 374. New York: John Wiley & Sons. 1924. \$4.

² SMITH, G. M., OVERTON, J. B., GILBERT, E. M., DENNISTON, R. H., BRYAN, G. S., and ALLEN, C. E., *A textbook of general botany*. 8vo. pp. x+409. figs. 321. New York: Macmillan Co. 1924.

plants, the last under the following subtitles: crop plants, plants used in medicine, forestry and forest products, weeds, and plant diseases.

The book is a very suggestive one for teachers, and is a valuable contribution to the problem of presentation.—J. M. C.

Genetics

The recently published text on genetics by JONES³ is distinctly up-to-date, comprehensive, and practical. The thorough way in which recent literature is covered makes it extremely valuable as a reference book; and the practical flavor of the many impressive examples is a feature which should be of great advantage in agricultural schools. These very features might render it somewhat cumbersome as an elementary text, where the teacher plans to lay a foundation of pure theory at the beginning; but the author has in mind the agricultural student, and makes theory subservient to practice.

Chapter I sketches the rôle of genetics in agriculture, depicting its possibilities and its limitations. Chapter II discusses the influence of environment, emphasizes the necessity of distinguishing effects of environment from those of heredity, and treats in a practical way the question of inheritance of acquired characters. Chapter III explains why bisexual reproduction brings greater variation, and points out the advantages which result. Chapter IV presents, in a rather concentrated form, monohybrid and polyhybrid breeding results, together with examples of all types of factor interactions. Chapter V discusses the chromosome mechanism, linkage and crossing over, multiple allelomorphs, lethals, semi-lethals, and balanced lethals. Chapter VI takes up sex and sex-linkage, with the conclusion that sex control is hopeless. Chapter VII classifies variations from several points of view. "All mutations may ultimately be understood as the results of an orderly process." Mutations are so rare as to be relatively unimportant in the practical business of plant and animal improvement, as compared with the possibilities of recombining existing hereditary units through hybridization. Chapter VIII puts practical statistical methods in a readily available form. Chapter IX provides a thorough discussion of the efficacy of selection within populations of various types. Chapter X describes, by means of numerous examples, the part that has been played by hybridization in the production of cultivated plants and domesticated animals. Chapters XI and XII cover the same field as "Inbreeding and outbreeding."⁴ Some more recent evidence is added, but the previous interpretation remains unmodified. Nowhere has the reviewer encountered a more comprehensive treatment of the subject of sterility than appears in chapter XIII. All types of sterility are presented, including a thorough discussion of species and genus hybrids. Chapters XIV and XV furnish a very fitting conclusion; they contain specific recommendations,

³ JONES, D. F., *Genetics in plant and animal improvement*. 8vo. pp. viii+568. figs. 229. New York: John Wiley & Sons. 1924. \$5.

⁴ EAST, E. M., and JONES, D. F., *Inbreeding and outbreeding*. Philadelphia. 1919.

as to technique and general breeding program, for the improvement of all our more important agricultural types.

The text is illustrated by 229 figures and numerous tables. It carries a glossary of 114 terms and a bibliography of 312 titles.—M. C. COULTER.

Desert conditions

As plants constitute the most important part of animal environment, it is not surprising that a recent volume dedicated to desert animals⁵ should have not less than half its space devoted to plants and to conditions that limit their development. In discussing the factor of water, emphasis is placed on the fact that not only is precipitation decidedly limited, but it is usually liable to very great variation from monthly and annual averages. Thus average rainfall is not an accurate indicator of desert conditions, because a large proportion comes as non-available moisture, or because much of it falls in the cold season, and because from year to year the precipitation deviates widely from the normal.

In considering desert temperatures, attention is directed to the wide yearly and daily range. Instances of variations, such as from -0.5° to 37.2° C., and from 3° to 28° C. within 24 hours are cited. Surface soil exposed to the sun may show temperatures as much as 20° C. above that in the shade, with such maxima as 60° C. in Palestine, 84° C. on the Loango Coast, and 78° C. in the Sahara. Other climatic factors are well summarized, and this summary, considered along with the condition of soil and water courses, is so presented as to form a good picture of desert conditions.

In addition to the food relations of plants and animals, considerable space is devoted to the shelter afforded animals by certain plants. Thus two woodpeckers are found most abundantly within the range of the giant cactus, *Cereus giganteus*, in whose stems they roost. The small elf owl is found exclusively in deserts where this cactus grows, using the abandoned woodpeckers' holes for its nests. Several other birds are somewhat less dependent upon the cactus and its woodpecker holes, but have an extended range where these occur.

The chapters on the physical environment of desert animals and on their colors are interesting, and go to make the book an important one in bringing graphically before the reader a picture of desert conditions. Well chosen illustrations add to the interest of the volume.—GEO. D. FULLER.

Transpiration and sap ascent

Plant physiologists, to whom DIXON's monograph has long been the classic work, will welcome the small volume⁶ recently published which brings up-to-date the work in this field. The first of these three lectures, delivered before the University of London, treats of the criticisms (adverse and favorable) which have

⁵ BUXTON, P. A., Animal life in deserts. A study in the fauna in relation to the environment. London. 8vo. pp. vii+176. figs. 43. 1923. Edward Arnold & Co. 10/6.

⁶ DIXON, H. H., The transpiration stream. London: Univ. of London Press. pp. 80. 1924.

been offered of the theory of a continuous column of water in the tracheae. The researches of HOLLE, BODE, URSPRUNG, RENNER, and NORDHAUSEN are reviewed, and the conclusion is reached that the cohesion theory stands to-day more firmly supported by experimental evidence than ever before.

In the second lecture DIXON discusses the work of BOSE, and records his inability to obtain the same results with the same methods. The more important work of URSPRUNG and BLUM on the living cells of the vascular system is also considered quite carefully, and it is concluded that as yet there is no need of assuming the intervention of any vital cell processes in the ascent of sap.

The third lecture constitutes a valuable summary of the very considerable experimentation of the lecturer toward the solution of the problem of the movement of organic substances in the plant. Naturally only such other researches are considered as have a direct bearing on the author's own theory of the transport of organic matter by the transpiration stream, but they are marshalled in formidable array. In the preface, however, a moderate position is assumed, and the outstanding need presented is not the acceptance of this new view, but renewed experimentation to ascertain the truth.

The bibliography of over sixty titles serves to make this truly a supplement which bridges over the decade since the publication of the monograph, and brings together all the literature on transpiration and sap ascent up to the past year.—H. S. WOLFE.

Regeneration

At the time of his death, LOEB had in the hands of his publishers a small book on regeneration⁷ which will probably stand as his final, but certainly not his greatest contribution to biology. The book deals with the problems of regeneration in *Bryophyllum calycinum*, a plant which had been a favorite subject of research with the author for a number of years. The discussion is divided into two parts, the first of which considers the problem of mutilation and regeneration, and the second deals with the factors concerned in polarity of regeneration.

The general conclusions reached from the eight short chapters in part I is that a mass relation can be used to explain the amount of regeneration that occurs. Stated briefly, this mass relation is that equal masses of isolated tissues of the same type (as sister leaves or pieces of stem) under equal conditions of light, temperature, etc., produce about equal masses of regenerated roots and shoots in an equal length of time. The inhibiting action of the growth of one group of bud anlagen upon another group is found in the fact that sap and nutrients start to flow to the group that starts growth, and the continuation of this flow to the growing organs prevents the other anlagen from obtaining the nutrients and sap necessary for growth initiation. Gravity also affects the course of regeneration by causing the nutrients to percolate to the lower edge of a leaf suspended vertically on edge, so that the lower anlagen received the nutrients brought down by gravitation.

⁷LOEB, JACQUES, *Regeneration*. Pp. xii+143. New York: McGraw-Hill. 1924.

With reference to polarity, two possibilities are recognized, first that there might be a chemical difference between the ascending and descending sap which determines the nature of growth, and second, that the anlagen reached by ascending and descending sap are primarily different. LOEB decides in favor of the second alternative, since it was possible to secure regeneration of roots with both ascending and descending saps, and certain mass relations tended to corroborate this view.

The student of regeneration will be struck by the rather naïve and superficial experimentation and philosophy which the author has developed in this book. It seems to the reviewer, indeed, that the book is much more a contribution to the subject of nutrition and growth than to an understanding of regeneration. The main conclusion, that it requires a definite amount of nutrients to grow a definite amount of dry matter, is too obvious to need a book to set it forth. The observations recorded and the philosophy developed leave us about as much in the dark as ever upon the fundamental problems of regeneration.—C. A. SHULL.

The ferns

BOWER⁸ has begun the presentation of the Filicales in the series of Cambridge Botanical Handbooks. This first volume deals with an "analytical examination of the criteria of comparisons," and is a detailed presentation of the facts and conclusions which he has been developing in previous publications. The subjects discussed will give a good idea of the contents. They are as follows: life history, habits and habitat, theoretical basis for systematic treatment, morphological analysis of shoot system, leaf architecture, cellular construction, vascular system of axis, vascular system of leaf, size a factor in stelar morphology, dermal and other non-vascular tissues, spore-producing organs (sorus and sporangium), gametophyte and sexual organs, embryo, abnormalities of life cycle, organographic comparison with other plants.

As may be inferred from this list of subjects, the volume is a treasure house of facts concerning the structure of Filicales, accompanied by suggestive conclusions. It certainly should be consulted by all morphologists. The theory of the "primitive spindle" is applied in the interpretation of structures, showing that filamentous structure is inherent in embryology, although frequently disguised. The conclusion is that such considerations "throw some welcome light upon the general architecture of the plant body, and point in the direction of a filamentous origin for even the most complex sporophytes."—J. M. C.

New Zealand plants

COCKAYNE⁹ has given, in a very concise and instructive form, the results of his personal experience of 35 years in the cultivation of the native plants of New

⁸ BOWER, F. O., *The ferns (Filicales)*. Vol. I. Cambridge Botanical Handbooks. 4to. pp. 359. figs. 309. 1923.

⁹ COCKAYNE, L., *The cultivation of New Zealand plants*. Auckland: Whitcombe and Tombs.

Zealand. The number of plants included exceeds 800. The habitat, or "place of dwelling" as COCKAYNE calls it, has been given special attention. There are 24 excellent photographs, a map, and a very attractive frontispiece representing the "crimson Manuka" (*Leptospermum scoparium* var. *Nichollsii*). The book deals with the methods of procuring and cultivating the plants, and has chapters on trees, shrubs, and climbing plants suitable for gardens, also on ferns for the open garden, plants for house decoration, native plants for school grounds and children's gardens, and town gardening. One paragraph may be quoted:

"Though the wild New Zealand plants live in all kinds of situations, and thrive under conditions unknown in gardens, it must not be thought that the majority are not amenable to cultivation. Quite the contrary is the case; only a small minority refuse to change their abode. Yet there is a deep-seated conviction to the contrary, notwithstanding the fact that many species have been cultivated for years."—R. B. THOMSON.

MINOR NOTICES

Shrubs of Indiana.—DEAM²⁰ has published a noteworthy contribution to the flora of Indiana, under the auspices of the Department of Conservation. The *Trees of Indiana* had been published previously, and developed a demand for a similar publication dealing with the shrubs. DEAM, as State Forester, has made a detailed study of the whole state, and the published results, therefore, are very complete. The classification, descriptions, and distribution are well presented, and in such a way that the non-professional user of the book may recognize the plants, especially helped by the numerous excellent plates.

It is the author's claim that the real value of shrubs is not appreciated. On this basis he divides them into three classes: those of ornamental value, those used in medicine and the industries, and those whose fruit is used. It is a credit to the Department of Conservation that it provides for the publication of such contributions.—J. M. C.

NOTES FOR STUDENTS

Gregarious flowering.—The simultaneous flowering at long intervals of many individuals of the same species has been recorded in many tropical and subtropical lands. The most remarkable record, extending (with some interruptions) back to 292 A.D., is that of a bamboo, *Phyllostachys puberula*, a native of China and Japan. All the individuals of this plant, however widely separated, have bloomed simultaneously every 60 years and then have died, and been replaced by seedlings. *Bambusa arundinacea*, of India, is known to flower simultaneously every 32 years, while some of the bamboos of southern Brazil are said to blossom at intervals of 13 years. SEIFRIZ²¹ has recently shown that a bamboo of the West Indies, *Chusquea abietifolia*, has a cycle of 33 years.

²⁰ DEAM, C. C., *Shrubs of Indiana*. Ind. Dept. Conservation Publ. 44. pp. 351. pls. 146. 1924.

²¹ SEIFRIZ, W., The length of the life cycle of a climbing bamboo. A striking case of sexual periodicity in *Chusquea abietifolia* Griseb. *Amer. Jour. Bot.* 7:83-94. 1920.

Although such periodicity has been attributed to drought stimulus, SEIFRIZ¹² presents evidence that this hypothesis is without supporting data, and shows that it has been impossible to connect it with any climatic phenomena. Further, PETCH¹³ has shown that such simultaneous flowering does not characterize all bamboos, although it may include species other than those cited. In particular he presents data to show that the giant bamboo, *Dendrocalamus giganteus*, does not flower gregariously.

A somewhat similar case is described by both SEIFRIZ¹⁴ and PETCH (*loc. cit.*) in the simultaneous flowering of the palm *Corypha umbraculifera*. On analysis by the latter author, it would seem that these palms reach a condition of maturity in 37 to 43 years, and that in Ceylon it is common to have groups of several or many individuals flower simultaneously every few years.

Another still more remarkable instance is recorded by PETCH of the gregarious flowering in Ceylon of several species of *Strobilanthus*, small trees of the Acanthaceae. They cover large tracts in the hill country, where several species flower and die simultaneously at intervals of 12 years. The different areas occupied by these plants may be arranged in two series, each maintaining its 12 year cycle, but with an interval of 5 (or 7) years between the times of general flowering and death of the two series. This has caused the erroneous assumption that *S. sexennis* flowers every 6 years. The situation is made still more complex by the behavior at Peradeniya of *S. viscosus*, which has flowered at intervals of 8 years, and it has been quite impossible to connect any of the flowering of *Strobilanthus* with any external factors.

The pigeon orchid, *Dendrobium crumenatum*, seems to present a still more complicated situation, for it has been observed to flower gregariously at very irregular intervals, but without the death of the plants after flowering. A record extending over a period of 4 years gave 27 flowering periods occurring at intervals varying from 10 days to several months. Recent investigations by SEIFRIZ¹⁵ at Buitenzorg, Java, confirm the conclusions formerly reached by BURKILL, from data obtained in the Straits Settlements, that the flowering always came 8 days after a heavy rainfall. This gregarious flowering of orchids differs from the other instances cited, since it involves individuals of quite different ages, and seems to be the only case in which such flowering has been related to external stimulus.—GEO. D. FULLER.

¹² SEIFRIZ, W., Observations on the causes of gregarious flowering in plants. Amer. Jour. Bot. 10:93-112. *pl. 1*. 1923.

¹³ PETCH, T., Gregarious flowering. Ann. Roy. Bot. Gard. Peradeniya 9:99-117. 1924.

¹⁴ SEIFRIZ, W., The gregarious flowering of the tailpot palm, *Corypha umbraculifera*, at Peradeniya, Ceylon. Bull. Torr. Bot. Club 51:341-350. *pls. 11*. 1924.

¹⁵ ———, The gregarious flowering of the orchid *Dendrobium crumenatum*. Amer. Jour. Bot. 10:32-37. 1923.

Taxonomic notes.—RYDBERG¹⁶ has published the result of his studies of *Odontotrichum*, recognizing 35 species, 6 of which are described as new.

BLAKE¹⁷ has described 13 new species of *Verbesina*, bringing the number of species in this genus to 185, making it far the largest genus in its subtribe, and, with the exception of *Bidens*, the largest in the tribe Heliantheae.

GRESS¹⁸ has published a very useful manual of the grasses of Pennsylvania. In the prefatory pages there are suggestions to amateur botanists, a statement of the genetic relationships of grasses, an explanation of their classification, and a full description of their gross anatomy. About 250 species and varieties are presented, in 70 genera, with abundant text illustrations to help in identification.

Recent contributions from the Gray Herbarium contain the following papers: PENLAND¹⁹ has presented a very detailed account of the North American species of *Scutellaria*, recognizing 17 species, including several new combinations. FERNALD²⁰ has published 5 short papers, dealing with *Polystichum mohrioides* and other subantarctic or Andean plants in the northern hemisphere; the dwarf Antennarias of Northeastern America (11 species, 2 of which are new); the eastern representatives of *Arnica alpina* (8 species, one of which is new); some species of *Senecio* of eastern Quebec and Newfoundland; and new or recently restudied plants of eastern America (including a new species of *Agoseris* from the Quebec region). ROBINSON²¹ has published some preliminary records to a general presentation of Eupatorieae. They include 2 new species of *Ophryosporus*, 16 of *Eupatorium*, and 11 of *Mikania*. In the same contribution JOHNSTON has published some new plants of Portuguese West Africa, 12 species in as many genera. The same author considers certain South American Proteaceae (one new species), and also presents a continuation of his studies of the Boraginaceae, defining and describing the "Old World genera" (60 in number), among which 4 new genera are established (*Microcaryum*, *Amblynotus*, *Oreogenia*, *Chionocharis*). He also publishes notes on miscellaneous American Boraginaceae.

FASSETT²² has investigated the two species of *Zizania* (*Z. palustris* and *Z. aquatica*) recorded in GRAY's *Manual*, with the following result. The species are *Z. aquatica* with three varieties (2 of them new) and *Z. latifolia*.

¹⁶ RYDBERG, P. A., Some Senecioid genera. II. Bull. Torr. Bot. Club 51:409-420. 1924.

¹⁷ BLAKE, S. F., New South American Verbesinas. Bull. Torr. Bot. Club 51:421-436. 1924.

¹⁸ GRESS, E. M., The grasses of Pennsylvania. Penn. Dept. Agric. 7:245. 1924.

¹⁹ PENLAND, C. W., Notes on North American Scutellarias. Contrib. Gray Herb. no. 71. 1924.

²⁰ FERNALD, M. L., *idem*, no. 72. 1924.

²¹ ROBINSON, B. L., *idem*, no. 73. 1924.

²² FASSETT, N. C., A study of the genus *Zizania*. Rhodora 26:153-160. 1924.

Z. palustris disappears as a variety of *Z. aquatica*, and *Z. latifolia* emerges from a confused synonymy.

NELSON²³ has published 4 new species of *Phlox*, 2 new genera of Compositae (*Eremohylema* and *Calhounia*), and 8 miscellaneous new species. The new genus *Calhounia* contains 14 species previously assigned to *Nocca* or *Lagascea*.

PAYSON²⁴ has published a new genus (*Scolioxon*) of Mexican Cruciferae; the species of *Pentstemon* native to Wyoming (33 in number); and also critical notes on various other groups.—J. M. C.

Photosynthesis.—A new method of estimating the amount of photosynthetic activity in marine algae has been employed by MOORE, WHITLEY, and WEBSTER²⁵ in a study of the influence of intensity and quality of light upon the rate of photosynthesis in green, brown, and red algae. As the bicarbonates are utilized in synthesis, the seawater becomes more alkaline. The degree of alkalinity change is easily determined by titration with phenolphthalein as indicator. The results they obtain by this method confirm the well-known facts that light from different regions of the spectrum from red to ultra violet has power to cause synthesis, but that the red rays are the most effective.

The distribution of the green, brown, and red algae in coastal waters is such that each kind is growing in the light intensity most favorable to the activity of its own chromophyll. Thus the green algae, with chlorophyll, are able to synthesize more rapidly than red algae when both are in strong light; but the red are more active than the green when both are in weak light. Moreover, the red forms synthesize carbohydrates more rapidly in weak light than when exposed to full sunlight. The brown forms are between the green and red in activity. Correspondingly each type is found growing at the depth which gives the light intensity most favorable to its own synthesis, the green algae near the surface, the brown somewhat deeper, and the red most deeply submerged. The red and brown pigments are held not to act as mere passive color screens, but as catalysts of photosynthesis in the same way as chlorophyll.

Studies of seashore pools show that the same changes in alkalinity occur in nature as in their experiments in vitro. The seasonal changes in hydroxyl ion concentration of the littoral waters may play an important part in the annual and seasonal development and succession of life in such habitats.—C. A. SHULL.

Permeability.—Using artificial cells as well as living and dried plant tissue, MACDOUGAL²⁶ has studied the permeability relations of the membranes. The

²³ NELSON, AVEN, Taxonomic studies. Univ. Wyoming Publ. Bot. 1:47-68. 1924.

²⁴ PAYSON, E. B., Miscellaneous papers. Univ. Wyoming Publ. Bot. 1:69-108. 1924.

²⁵ MOORE, B., WHITLEY, ED., and WEBSTER, T. A., Studies of photosynthesis in marine algae. Trans. and Proc. Liverpool Biol. Soc. 37:38-51. 1923.

²⁶ MACDOUGAL, D. T., The arrangement and action of material in the plasmatic layers and cell walls of plants. Proc. Amer. Phil. Soc. 63:76-93, figs. 2. 1924.

artificial cell was essentially the same as that used in previous investigations. It consisted of a cellulose extraction thimble infiltrated and coated with substances present in the living cell. Thus by the addition or subtraction of a substance, the effect of this substance on permeability could be studied. As the term permeability is used by MACDOUGAL, a decrease in permeability denotes a change in the colloidal condition of the cell wall and plasmatic membranes, so that only water and the more active ions pass through, while in cases of an increase in permeability the largest molecules pass through. The effects of the salts of the common bases on permeability were studied, and results were obtained substantiating RABER's conclusion that the relative density of the charge on the kation and anion of the salts is the thing to consider. Data concerning the effects of acids, bases, and salts on the swelling of agar and gelatin are given. Then various combinations of these substances and also pectin and lecithin were used in the artificial cell, and similar results obtained. For example, in the experiments on the effect of acids on the swelling or hydration of agar, increase in acidity caused decrease in hydration. A decrease in hydration means decrease in permeability, and in the case of the special cell, as the acidity increased the amount of endosmose or turgidity increased. Studies were made on the effect of acids, bases, and salts on the swelling of living and dried slices of *Opuntia*, the plasmatic masses of which have a rather high pentosan content. Finally, data on the effect of the concentration of the cell sap on the absorption of ions were recorded. The results obtained support the common idea that the osmotic concentration of the cell sap has little to do with the intake of ions.

Studies such as these of MACDOUGAL are doing much to increase our knowledge of the real nature of permeability.—S. V. EATON.

Hydrogen ion concentration.—Using colorimetric methods, ATKINS²⁷ has studied the hydrogen ion concentration of seawater. He finds that seawater may become as alkaline as P_H 9.7, due to very active photosynthesis. The maximum alkalinity was found in May and August, with a minimum in July. The maximum in May was found to correspond with the maximum average number of hours of sunshine daily, rather than with the maximum length of day, which would come of course in June. Data obtained from changes in the P_H value of the seawater are used to calculate the minimum amount of carbohydrates synthesized in the sea. The actual amount of carbohydrates found is the result of course of an equilibrium between production by photosynthesis and destruction by plant and animal respiration. It is stated that in a general way the P_H maxima may be correlated with the diatom maxima in early summer and autumn. Perhaps further work will show the P_H concentration a rather useful criterion in studying the distribution of algal life.—S. V. EATON.

²⁷ ATKINS, W. R. G., Hydrogen ion concentration of seawater in its biological relations. Pt. I. Jour. Marine Biol. Assn. 12:717-771. 1922.

———, Hydrogen ion concentration of seawater in its relation to photosynthetic changes. Pt. II. Jour. Marine Biol. Assn. 13:93-118. figs. 11. 1923.

Determination of chlorides.—LAWRENCE and HARRIS²⁸ have developed a method of determining the chloride content of plant tissue fluids which seems accurate and rapid. Essentially the method consists of the use of a known excess of silver nitrate to precipitate out the chlorides, the destruction or altering of the proteins or other organic compounds which might fix a certain amount of the silver by boiling the fluids with concentrated nitric acid, and finally the titration of the excess silver nitrate against potassium thiocyanate solution, ferric alum being used as an indicator. The nitric acid has the effect also of decolorizing the fluids and of freeing any chlorine that may be in organic combination. Writers on technique quite generally warn against the titration of the excess silver in the presence of the silver chloride precipitate, but the authors show that in the presence of nitric acid the silver chloride forms rather large masses, so that on account of the small surface exposed the reaction between the silver chloride and the potassium thiocyanate is negligible. In this way there is eliminated the most time-consuming part of the method as it has usually been practised.—S. V. EATON.

Light requirement of trees.—Using as a standard the amount of electricity generated by the light falling on a thermo-couple and measured by a sensitive galvanometer, BURNS²⁹ has determined the minimum light requirement of several forest trees more exactly than has previously been done. He used two strong nitrogen filled electric bulbs as a source of light, and secured differences in exposure by varying the distance between the light and the trees. Young trees 18–24 inches in height were used inclosed in bell jars, and the minimum light requirement was regarded as that which gave a respiration-photosynthesis coefficient slightly above unity, as determined by gas analysis. The minimum light requirement determined for 14 species was: *Pinus ponderosa* 306, *P. sylvestris* 287, *Thuja occidentalis* 186, *Larix laricina* 176, *Pseudotsuga mucronata* 136, *Pinus Murrayana* 136, *Quercus rubra* 133, *Celtis occidentalis* 115, *Picea Engelmannii* 106, *Pinus Strobus* 104, *Picea excelsa* 87, *Tsuga canadensis* 84, *Fagus grandifolia* 75, and *Acer saccharum* 34.—GEO. D. FULLER.

North American flora.—The fourth part of volume 24 contains a continuation of the Galegeae (Fabaceae) by RYDBERG, including 25 genera, with 117 species, 17 of which are described as new. The large genera are *Robinia* (20 species, 2 of which are new), *Benthamantha* (19 species, 8 of which are new), and *Diphysa* (17 species, 5 of which are new). The remaining 61 species are distributed among 21 genera.

²⁸ LAWRENCE, J. V., and HARRIS, J. A., Tests of a wet oxidation and modified Volhard method for the determination of chlorides in plant tissue fluids. Jour. Amer. Chem. Soc. 46:1471–1477. 1924.

²⁹ BURNS, G. P., Studies in tolerance of New England forest trees. IV. Minimum light requirement referred to a definite standard. Vermont Exp. Sta. Bull. 235. pp. 32. 1923.

The ninth part of volume 7 contains the conclusion of Aecidiaceae (Uredinales) by ARTHUR. Four genera are presented: *Uredo*, with 60 species (2 new); *Aecidium*, with 84 species (4 new); *Peridermium*, with 7 species (2 new); and *Caeoma*, with one new species. It is interesting to note that the author's name is associated with 32 species of *Uredo* and 43 species of *Aecidium*. There are 20 pages of additions and corrections to Uredinales, including a new genus (*Synomyces*) based on *Coleosporium Reichei* Dietel.—J. M. C.

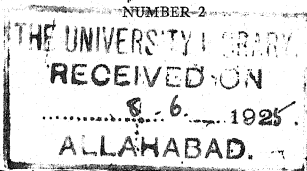
Embryogeny.—SOUÈGES,³⁰ in continuation of his unremitting work on the embryogeny of angiosperms, has published an account of *Sagina procumbens* (Caryophyllaceae), with interesting conclusions. He has also published, in the Proceedings of Académie des Sciences for 1924, abstracts of his results with the following forms: *Polygonum aviculare* (January), *Poa annua* (March), *Linum catharticum* (April), *Sherardia arvensis* (June), *Sparganium simplex* (July), and *Euphorbia Esula* (November).—J. M. C.

North American Flora.—The fourth part of volume 10 continues the presentation of the Agaricaceae, including *Inocybe* by C. H. KAUFFMAN and *Pholiota* by L. O. OVERHOLTS. *Inocybe* includes 105 species, 16 of which are new; and *Pholiota* includes 48 species, 2 of which are new. It is interesting to note that 28 species of *Inocybe* are credited to PECK, and 20 species to ATKINSON. In *Pholiota* 19 species are credited to PECK.—J. M. C.

Morphological studies of Scrophulariaceae.—HOLM³¹ has published a very detailed account of the anatomical structure of the roots, stems, and leaves of *Ilysanthes*, *Scrophularia nodosa* var. *americana*, and *Linaria canadensis*, especially of the two latter species. The details are too numerous to mention, but such data are very valuable for comparative studies of responses and relationships.—J. M. C.

³⁰ SOUÈGES, R., Développement de l'embryon chez le *Sagina procumbens* L. Bull. Bot. France IV. 24:590-614. figs. 48. 1924.

³¹ HOLM, THEO., *Ilysanthes*, *Scrophularia*, and *Linaria*; a morphological study. Amer. Jour. Sci. V. 8:395-410. 1924.



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STONY LAYER IN SEEDS OF GYMNOSPERMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 333

EDUARDO QUISUMBING

(WITH PLATE XI AND EIGHTY-EIGHT FIGURES)

Introduction

A critical study of the stony layer in seeds of gymnosperms is desirable from the standpoint of comparative morphology, phylogeny, and chemistry. There is an extensive literature, but many problems are still unsolved, especially those concerning the chemistry of the stony layer and the rôle it plays in the interpretation of adjacent tissues. The failure to study this layer from its early stages to maturity has caused disputes regarding its nature, and has led to erroneous interpretations of the surrounding tissues, especially the integument. In most of the genera of gymnosperms the stony layer has been mentioned or partially described; but a lack of appreciation of the importance of the layer, or the real difficulty of obtaining material and the exceptionally difficult technique have prevented a complete study.

The earliest stages in the development of the ovule have not been studied fully in any of the genera of Cycadales, Ginkgoales, Coniferales, and Gnetales, and publications dealing with young stages have failed to note the nature of the integument in its earliest stages. The difficulty in securing very young stages is doubtless partly responsible for our meager information, especially in case of

the cycads. Taking advantage of the exceptional facilities afforded at the University of Chicago, I have studied the history of the stony layer in some of the gymnosperms, with the hope of filling part of the gap in our knowledge.

OLIVER and SCOTT (41) studied the seed of *Lagenostoma Lomaxii*; STOPES (58, 59) investigated several genera of cycads; CHAMBERLAIN (12) studied the stony layer in *Dioon edule*; SPRECHER (57) investigated *Ginkgo biloba*; WIELAND (71, 72, 73) described the seeds of Bennettitales; and several others have been interested in one stage or another in the development of the ovule and the seed.

It is the purpose of this paper to present as complete an account as possible of the origin and development of the stony layer of the seed in several gymnosperms; to discuss the homologies of the stony layer in the different groups; to use the stony layer in interpreting the nature of the integument; and to touch upon the complex problem of the chemistry of lignification of stone cells.

Because the stony layer in these seeds is of integumentary origin, as far as the integument extends, a very critical study of the integument is of paramount importance. It is not the writer's intention to devote much time to the homologies and interpretations of the tissues covering the ovules, nor to the nature of these tissues, as this subject has been amply discussed.

The paper deals with *Lagenostoma*, some species of *Cycas*, *Ginkgo*, *Pinus*, *Juniperus*, *Torreya*, and *Gnetum*; with a glimpse of the stone situation in some angiosperm seeds. Unfortunately, because young *Lagenostoma* specimens were not available during the progress of this work, reference will be made only to the works of WILLIAMSON (75, 76), BUTTERWORTH (10), OLIVER and SCOTT (40, 41), ARBER (2), CHODAT (14), and PRANKERD (46).

This work was undertaken at the suggestion of Professor CHARLES J. CHAMBERLAIN of the University of Chicago, to whom I wish to express gratitude for his valuable suggestions and criticism, and also for the privilege of using his slides of cycads and preserved material.

History

So far as I am aware, the first reference to the stony layer in seeds of gymnosperms was made by MIQUEL (34) in 1842. His

description of the *Cycas revoluta* seed was taxonomic, but it was an attempt to distinguish the stony layer from the rest of the tissues surrounding it. Three years later (35) he studied *C. revoluta* and *C. circinalis*; and still later (36) *C. inermis*. Although brief and primarily taxonomic, this account is of great value morphologically, as he gave a description of the integuments and their nature. He recognizes two integuments. In his earlier papers he mentions one integument composed of three layers. He believes that the outer integument in the case of *C. inermis* is composed of two intimately associated layers, an outer fleshy and an inner which lignifies, so that the stony layer originates from the inner layer of the integument. Evidently he did not study young stages of the ovules or he would not have assumed a dual nature of the integument. For the same reason other botanists have reached erroneous interpretations of the integument of cycads.

Before MIQUEL, BROWN (9) in 1826 gave an account of the structure of the ovule and the female flower of the cycads, with a history of the various opinions concerning the female flower and the ovule. Others following him in studying the nature of the female flower and the ovule are as follows. GRIS (23) compared the ovules of *Cycas* and *Zamia* with those of *Ricinus*. WARMING (70) in his researches on cycads described the structure of the seed. His finding of a thick fleshy layer on the inner side of the stony layer of the integument is interesting, as this layer had been overlooked by early observers. KERSHAW (26), who made a study of the structure and the development of the ovule of *Bowenia spectabilis*, finds a general structural agreement with the seeds of the Medullosae, *Trigonocarpus*, *Stephanospermum*, and *Cardiocarpus*. The topography of the ovule is already well known in all genera of cycads.

Next to the nature of the female flower and the ovule of the cycads, the integument is the most debated structure, some writers believing it to be single, while others think it has a dual nature. Much of the uncertainty has arisen from the failure to study the integument in its earliest stages.

MIQUEL (34, 35, 36) found that in the ovule of *Cycas revoluta* the integument is made up of three layers, and later published observations on the ovule and integument of other cycads. He described an outer fleshy layer of the integument, composed of regular paren-

chymatous cells with abundant mucilage and brownish substance which we now know to be tannin; within this a layer of lignified or bony cells which become thickened with age; and he recognized a layer between the stony coat and the endosperm. Six years later (36) he described the ovule and integument of *C. inermis*, changing somewhat his views regarding the nature of the integument, for he recognized two integuments, which he called internal and external integuments. His early works described the ovule as covered by one integument composed of three layers, but in his latest work he recognizes two integuments, the outer integument consisting of two layers, an outer fleshy and an inner lignified. This might suggest a possible origin of the stony layer. Evidently he did not study the young ovules, because he distinguished at the very beginning a distinct differentiation of the intimately associated layers in the outer integument.

BROWN (9) found only one envelope in the ovule of *Cycas*, but GRIFFITH (22) concluded that it consists of two united throughout their whole extent. GRIFFITH evidently examined only mature ovules, where this condition is very pronounced (fig. 27), the demarcation between the stony layer and outer fleshy layer in *Zamia* being very sharp; but the nature of the integuments cannot be interpreted from the older stage. GRIS (23), investigating the ovules of *Cycas* and *Zamia*, stated that the female reproductive organ is a naked ovule, and that the envelope which covers it is an integument and not an ovary. OUDEMANS (43), in reviewing and commenting on the subject of integument, stated that the ovule of cycads is provided with one envelope only, but composed of two layers. BRAUN (8) mentioned the origin of the integument, and considered that it arises from the nucellus. PEYRITSCH (45) is of the same opinion.

BERTRAND (7), in his work on the integuments of the seeds of gymnosperms, gave a good account of the morphology of the stony coat and described the origin of its isodiametric cells. According to him the integument of Cycadaceae is composed of two parts, a fleshy part and a ligneous one. He further distinguished two layers in the stony layer, one composed of isodiametric cells and the other of elongated cells. With reference to the origin of the stony layer

he had the same idea as MIQUEL. The development of the integument was studied by WARMING (69), who believed a single integument arises from the fusion of two original integuments. WORSDELL's paper (78) on the morphology of the sporangial integuments reviewed the situation, and concluded that the ovule has a single integument. CELAKOVSKY, however, considers it along with *Cephalotaxus* as having two integuments intimately united, the outer fleshy and the inner woody. STOPES' account (58) of the vascular anatomy of the sporophylls and ovules of all the genera included a discussion of the integument. She described briefly the three layers, and claimed a double nature of the integument in cycads, basing her assumption upon the bundle situation in the *Lagenostoma* cupule and in *Cycas*. Later (59) she published a paper dealing with the origin of the stony layer and the morphological nature of the sarcotesta, which she regards as the homologue of the cupule of *Lagenostoma*, so that the envelope of the cycad ovule is double, the sarcotesta being the outer integument. In 1906 WORSDELL (79) agreed with the views of CELAKOVSKY regarding the nature of the integument. He thinks that there are two integuments in the ovule of cycads, and calls the outer fleshy layer the outer integument, and the inner fleshy layer with the stony layer as together constituting the inner integument. He also suggests homologies of the so-called outer integument with the detached cupule of *Lagenostoma* and *Neuropteris heterophylla*.

KERSHAW (26), after studying the structure and development of the ovules of *Bowenia spectabilis*, doubts the views offered by STOPES. SALISBURY (51) infers that the duality of the integuments in cycads is unlikely on account of the homogeneous development of the integument of *Bowenia* observed by KERSHAW, and more especially the anatomy of *Trigonocarpus shorensis* on which the paper is based. She states that such inner flesh as is present is simply the unsclerized internal lining of the hard shell facing the free nucellus. CHAMBERLAIN (12), in his work on the ovule and female gametophyte of *Dioon edule*, made a comprehensive study of the three layers of the integument, and also reviewed previous work on the subject. SCOTT (52) expresses some doubt regarding the suggestions made by STOPES that the cycad testa is essentially

double; and COULTER and CHAMBERLAIN (16) express the same opinion. DE HAAN (18) made a comparative study of the morphology and phylogeny of the ovule and its integuments, but gave no detailed descriptions.

Although several botanists mentioned and figured occasional early stages in the development of the integument, no one has given a complete series of stages showing the origin, development, and nature of the stony layer in any of the cycads. WARMING (68) studied *Cycas circinalis* and *Ceratozamia longifolia*, and TREUB (65) gave a description of the development of the ovule in *Ceratozamia longifolia*, in which one of his figures indicates that the integument might be homogeneous before it differentiates into the outer fleshy and inner fleshy layers. He also showed and sketched the stony layer of older ovules. Later LANG (29) investigated the ovule of *Stangeria paradoxa*. He described briefly the three layers in the integument which are distinguishable while the young megaspore is still uninucleate. His fig. 2, a photograph, shows the undifferentiated integument. Two later stages are photographed, but the sections were evidently thick and probably rather deeply stained, so that, in the absence of any detailed description, the course of development is uncertain. STOPES (58, 59) studied the development of the stony layer after the three layers are formed, and speculated upon the origin of the stony layer. She admits the intimate connection of the innermost cells of the outer fleshy and outermost part of the stony layer, but could not believe it to belong to the outer fleshy. CHAMBERLAIN (12), in his work on *Dioon edule*, although he did not study the development of the integument, suggested that the integument is homogeneous in character at an early stage. SMITH (56) studied the development of the ovulate strobilus and ovule of *Zamia floridana*, but was concerned only with the megaspore mother cell and the surrounding tissues, so that, while she had the necessary stages, she did not study the integument except to note that it is hypodermal in origin. The growth of the nucellus and the integument was studied by KERSHAW (26) in *Bowenia spectabilis*, but her description of the integument and the stony layer was incomplete.

Ginkgo has been the subject of much investigation and conjecture

for a long time. While the taxonomy, the vascular anatomy, the sperms, etc., have attracted the attention of botanists, the ovule has likewise been regarded as important. The *Ginkgo* ovule offers not only material for more critical study about its organization, but also a subject for comprehensive comparative studies. The taxonomic position of this genus has also been the subject of much speculation. The accounts are so extensive and numerous that no effort will be made to narrate them here. In conjunction with the taxonomy of the genus, the morphology of the female flower has occasioned much dispute. Many botanists, as RICHARD, BRONGNIART, SCHLEIDEN, and PARLATORE have contributed their observations and theoretical views to elucidate the morphology of the female flower. VAN TIEGHEM (66) regarded the collar of the ovule as a rudimentary arillus. Previously there were several who discussed the nature of the female flower. STRASBURGER (60) described the ring or collar at the base of the ovule as the rudiment of the first pair of leaves of a secondary shoot; EICHLER (19) called the collar an outer integument; and WILLIAMSON (76) stated that the aril or collar at the base of the ovule suggests a vestige of the completely investing cupule of certain seeds like those of *Lagenostoma*. STRASBURGER (61) changed his views and called the collar an arillus, and regarded the fleshy covering as an integument. EICHLER modified his view (20) and called it a rudimentary carpel. FUJII (21) summarized the different opinions regarding the morphology of the female flower, and observed that the collar was found to pass gradually into the lamina of the modified leaf. LANG (29) in his paper on *Stangeria paradoxa* referred to the analogy of the integument at early stages to that of *Ginkgo*. SEWARD and GOWAN (54) gave a history of the genus from its first description. They found in connection with the ovule that each is inclosed by a single integument, and at the base by an envelope or collar homologous with the lamina of the leaf. CAROTHERS (11) observed the homogeneity of the integument in the very young stages. SPRECHER'S (57) book on *Ginkgo biloba* gave a résumé of the different views regarding the nature of the ovule. He studied the very young ovule and its development until it became mature. He believed that there is but one integument differentiated into

three layers. He also studied lignification. SHAW (55) tentatively suggested in his paper, based on the anatomy, that the collar of the ovule is better regarded as a vestigial cupule, and expressed a view of the double nature of the integument. AFFOURTIT (1) reviewed again the history of the subject of the female flower, and also included a comprehensive discussion of the ribbing of seeds. COULTER and CHAMBERLAIN (16) gave a brief résumé of the views as to the nature of the cup or collar, and also the nature of the strobilus, and state that the integument is homogeneous at first, but by the last of May three distinct layers have become differentiated.

The nature of the stony layer was studied in passing by several authors, and none of them seem to agree on certain technicalities. The failure of many of them to mention the way the cells were cut resulted in an erroneous interpretation of the true nature of the stony layer. The plane of cut of the stony layer gives some difference in the appearance of the layers. Notable among the investigators who distinguished three layers was DE HAAN (18), but his description is so meager and incomplete that one cannot be sure whether he is describing the cross-section or the longitudinal section. BERTRAND (7) recognized but two layers of the stony layer, the outermost composed of small isodiametric cells, while the cells of the interior are slightly elongated longitudinally. SPRECHER (57) studied the development of the stony layer, but gave no detailed descriptions. His figures 149, 150, and 151, although rather diagrammatic, are suggestive.

The true nature of the female flower of *Pinus* has been an important question among botanists since the first announcement of their gymnospermous character by ROBERT BROWN (9) in 1826. He gave the early history of the subject, and included the works of LINNAEUS, GISKE, TREW, JUSSIEU, LAMBERT, SCHKUHR, SALISBURY, MIRBEL, SCHOUBERT, BAILLON, and several others.

Next in importance to the female flower is the ovuliferous scale, called by MASTERS (33) "fruit scale." There is a great diversity of opinion which has given rise to a voluminous literature. As the subject requires an extensive discussion, and as it is beyond the bounds of the writer to settle the controversy, reference will be made

only to the different opinions. The various claims have been fully outlined by MASTERS. RADAIS (47) and WORSDELL (74) made a critical review of the whole situation, and COULTER and CHAMBERLAIN (16) summarized the numerous different views.

The female flower of *Juniperus* has been discussed almost as much as that of the Cycadales, Ginkgoales, and other Coniferales. LOTSY (32) described when the genus was first established and the systematic work done upon it. Before him JACK (24) cited several who monographed the genus and studied its fructification. Studies on periodicity of this fructification were given by several botanists, and JACK himself contributed, but in most cases the reports were based on herbarium material rather than upon field and laboratory observations. Following such observations and studies, the most difficult question was about the true nature of the female flower.

VON MOHL (67) regarded the ovules as metamorphosed bracts, and SACHS (50) adopted the same view. JACK suggested that they were "scales." BAYER (3) made a critical study of the female flower of *Juniperus*, reviewed the subject, and concluded that the ovules are borne on sporophylls. RENNER (40) also reviewed the various interpretations and confirmed the findings of BAYER. Others who have contributed are RENNER (48), KUBART (27), NOREN (38), OTTLEY (42), NICHOLS (37), LOTSY (32), COULTER and CHAMBERLAIN (16), and DE HAAN (18).

The literature on the organography of the female flower of *Gnetum* is very extensive on account of the important position of Gnetales. Consequently only a brief account can be given here. The classic studies on Gnetales were started when *Gnetum* was first described. So far as I am aware, JUSSIEU was the first one to describe the genus in a taxonomic account in 1789. Many botanists, as RICHARD (1826), BROWN (1827), BLUME (1834), MEYER (1846), ENDLICHER (1847), BLUME (1848), LINDLEY (1853), AGARDH (1853), BENTHAM (1856), TULASNE (1858), and HENFREY (1859) have contributed their observations on the female flower. GRIFFITH (22) stated that the flower consists of a single naked ovule with two envelopes. He described also these envelopes, and although inferior as to accuracy, it is worth while noting, as it shows one of the earliest attempts to interpret the different tissues of the ovule. He

studied also the development of these envelopes in *G. scandens* principally, and other species, except *G. Gnemon*, and also the coat in the mature seed. The following have studied the Gnetales since then: HOOKER (1863), EICHLER (1863), DE CANDOLLE (1868), PARLATORE (1868), DE MONT and DESCAINE (1868), and VAN TIEGHEM (1869). STRASBURGER (60) gave a résumé of the various views on the female flower, and concluded that in *Gnetum* it is an adventitious bud. He also considered the three envelopes as three integuments; the inner ones he claimed to be homologous with the one internal integument of *Ephedra*, and the outer one an external integument. He also regarded the female flower as an ovary, with two carpels. BECCARI (4) studied the development of the female flower of *Gnetum Gnemon* and confirmed STRASBURGER's interpretation that the complete flowers have three envelopes, while the incomplete ones have but two, and that the integument surrounding the ovule does not belong to the ovule. After BECCARI, BERTRAND (7) also studied the ovule of *Gnetum*, but did not contribute much on the morphology of the female flower. STRASBURGER (61) abandoned the opinion he had advanced earlier as to the nature of the female flower, and called it an ovule. He makes comparison of the internal and intermediate envelopes of *Gnetum* with the internal integument of *Ephedra*, and the external integument of *Gnetum* with the external integument of *Ephedra*. KARSTEN (25) studied *Gnetum* and confirmed the findings of STRASBURGER and BECCARI regarding the nature of the envelopes. LOTSY (31), in his résumé of the previous work on the inflorescence and female flower of *Gnetum*, outlined the different views on the subject. He also gave a detailed description of the development of the integuments in the three species. He called the female flower an orthotropous ovule, with a single integument. This is contrary to STRASBURGER's (21) view of two integuments. He further stated that the integument is surrounded by two whorls of bracts, which he called an external and internal perianth, and concluded that the inner envelope is the only integument present. WORSDELL (78), studying the morphology of the sporangial integuments, called the outer envelope a perianth and the two inner ones integuments, and claimed that the two integuments occur uniformly throughout

gymnosperms. The writer's findings, however, do not agree with his last view.

BENSON (5), in seeking to connect the angiosperm with the gymnosperm flower, took *Gnetum* as the basis. She made homologies of the female inflorescence of *Gnetum* with those of *Castanea*. As regards the nature of the flower and the envelopes, she had some interesting views, although their real significance and application are doubtful. She thought the nucellus and the lobed inner integument equivalent to a synangium. She further stated that the female synangium of *Gnetum* is further surrounded by two envelopes, "the inner of which probably represents the outer integument of the angiospermic seed, and the outer, as has frequently been pointed out, the carpel." COULTER (15) made homologies of the three layers of the integument in Cycadophytes, Ginkgoales, and Coniferales with the integuments of *Gnetum*. BERRIDGE (6) called attention to certain resemblances mentioned by SEWARD between the seed of *Gnetum* and that of *Bennettites*, both as to external features and internal features. The most complete historical account of the various opinions concerning the inflorescence and the female flower of *Gnetum* was given by LIGNIER and TISON (30). THOMPSON (64) made a thorough investigation of *Gnetum*. As to the female flower, he concludes "that the strobilus of *Gnetum* is closely related to the catkins of the Amentiferae; that the flowers are reduced from a bisporangiate condition; that the inner envelope of the ovulate flower is an ovary homologous with that of the angiosperms and bearing a true style (the micropylar tube)." COULTER and CHAMBERLAIN (16) gave a short account of the interpretation of the female flower and also the outermost envelope. They called this structure the perianth, and the two innermost envelopes the two integuments. PEARSON (44) gave a complete history of the different views regarding the interpretation of the nature of the female flower of *Gnetum* and also in reference to the envelopes. He discussed fully the morphology of the female flower and also the nature of the envelopes. THODAY (63) gave a recapitulation of the structure of the female flower and followed the view advanced by COULTER and CHAMBERLAIN (16). She regarded the "perianth" of probably foliar nature. STRASBURGER (60) stated that when

there are but two integuments present in *Gnetum Gnemon* the order of the growth of the integuments is basipetal, but when the flower is perfect the condition of growth is from base to top. LOTSY (31) in his studies of *Gnetum* remarked that the three envelopes arise in a centripetal direction. COULTER (15), working on *G. Gnemon*, concluded that the two integuments develop in basipetal succession.

A summary of the number of integuments is not out of place. GRIFFITH (22) mentioned that there are three envelopes and that two of them belong to the naked ovule; he spoke of them as envelopes and not as integuments. STRASBURGER (60) called the three envelopes three integuments, but later (61) changed his view and called the outermost envelope a perianth and the two inner envelopes integuments. LOTSY (31) concluded that the ovule is provided with a single integument and the two outermost envelopes he designated as bracts, calling them the "internal and external perianth." COULTER (15), BERRIDGE (6), COULTER and CHAMBERLAIN (16), SEWARD (53), THODAY (63), and DE HAAN (18) maintained the view sponsored by STRASBURGER in 1879, that there are two integuments, and that the outermost envelope is the "perianth."

The stony layer has been described by many botanists, but in most cases the descriptions are incomplete and meager, and the illustrations are either too diagrammatic or inaccurate. GRIFFITH (22), describing the mature seeds of *Gnetum scandens*, called the outer envelope "baccate," the middle one "drupaceous," and the inner one "fibro-cellular." Judging from his descriptions, he recognized but two layers of the stony layer. He described the layer outside of the palisade region as "externally sulcate," the palisade layer as "cells arranged transversely," and the innermost fibrous layer as "a great number of the longitudinally disposed fibers, which are longer and much less in diameter than those of the outer baccate." COULTER (15), studying *G. Gnemon*, decided that the outer integument is differentiated into an outer fleshy and an inner stony layer. BERRIDGE (6), who suggested resemblances between the seeds of *G. Gnemon* and those of *Bennettites Morierei*, was able to distinguish but two layers belonging to the stony layer, the palisade and the fibrous. THOMPSON (64) described the outer integument as COULTER did, but gave more elaborate description of the outer

fleshy layer. Judging from these accounts and others of the same nature, it is evident that investigators have missed the outermost layer of the stony layer, which is made up of small isodiametric cells. THODAY (63) figured but two layers of the stony layer, a palisade layer and a row of isodiametric cells outside of the palisade layer.

Material and methods

The material used included some slides of *Lagenostoma* by Lomax; but the stages had little bearing upon the stony layer, so that I have depended upon the results obtained by OLIVER and SCOTT (40, 41).

In the cycads it is difficult to obtain a complete series in the same genus or species from very young stages until the ovules are ripe and the shell hard; and so some stages were studied in one genus and some in another; but the material was sufficient for a comparative study of the nature of the mature stony layer in various genera. The following genera are included in the study: *Cycas*, *Dioon*, *Stangeria*, *Microcycas*, *Ceratozamia*, and *Zamia*. In the Ginkgoales a very complete series of stages was secured, showing the development from the beginning of the integument until the stony layer became hard. In the Coniferales only three genera, *Pinus*, *Juniperus*, and *Torreya*, were studied, but the stages, especially in *Pinus* and *Juniperus*, were rather complete. In the Gentales only *Gnetum* is included in this paper, although a few stages in *Ephedra* and *Wehwitschia* were available.

While it is not intended at this time to describe the stony layer in the seeds of angiosperms, many genera of the lower Archichlamydeae, including *Carya*, *Corylus*, *Fagus*, *Hamamelis*, and *Juglans*, were collected and sectioned. For comparison, a brief mention will be made of only *Sassafras*, *Quercus*, and *Prunus*.

The histological technique for the younger stages is not very difficult; but when the stony layer begins to lignify, difficulties are encountered. The tannin or resin usually present in the integument hinders rapid penetration of the killing and fixing reagent, so that in most cases the ovules must be trimmed.

Some of the material was examined in the living condition, but most of it was sectioned with a microtome. Chromo-acetic acid

and hot corrosive sublimate acetic were used for fixing. For stages when the egg is already formed, the corrosive sublimate acetic proved to be the best; but for very young stages, when the female gametophyte is in the megaspore mother cell stage or in the free nuclear stage, chromo-acetic fixative is better. The specimens were left in the hot corrosive sublimate acetic for 10–15 minutes, and then transferred to 70 per cent alcohol. Otherwise CHAMBERLAIN'S (13) method was followed.

In the very early stages of the ovules no dissection or trimming was necessary. Those fixed in chromo-acetic generally floated, but this difficulty was overcome by means of an air pump. In dehydrating, 10, 15, 20, 30, 40, 50, 60, 70, 85, 95, and 100 per cent alcohol was used, and three changes were made a day. The mixture of absolute alcohol and xylol used was 2.5, 5, 10, 15, 25, 50, 75, and 100 per cent, with also three changes during the day. The 75 per cent xylol was colored with eosin, to prevent loss and to facilitate orienting the specimens previous to cutting. The material was left in xylol-paraffin for at least two weeks before imbedding. With the oven at 50–52° C., one hour was allowed, for very young ovules were infiltrated in 20–30 minutes, but later stages required about an hour.

A Spencer rotary microtome with Gillette blade was used for cutting. For older stages, especially those with fairly hard stony cells, an inclined holder was used. Serial sections, 5–10 μ in thickness, were cut at various angles. Mayer's egg albumen was used; this, however, was unsatisfactory after the stony layer began to harden. For these refractory stages Land's gum arabic fixative gave the best results.

The triple stain (safranin, gentian violet, and gold orange) was the most satisfactory with the orange dissolved in clove oil. Light green was also used, and in older stages gave good results. Iron-haematoxylin was used for very young stages, with a deep counter stain in gold orange.

Hard shells were softened by means of 100 per cent hydrofluoric acid. Ten days was sufficient for *Ceratozamia*, *Ginkgo*, and *Pinus*; *Gnetum* required 18 days, *Juniperus* 20 days, and *Cycas* two months.

Cellulose acetate (74) was tried to soften hard shells, but gave no satisfactory results.

Cycadofilicales

LAGENOSTOMA

The large group of fossil seeds of Cycadofilicales has been worked thoroughly by English investigators and several others; and Bennettitales have been investigated, especially by WIELAND (71, 72, 73). Using as types *Lagenostoma* for the Cycadofilicales and *Cycadoidea* for the Bennettitales, attention was confined to *Lagenostoma*, because in both genera the seeds are incased in an outer palisaded layer, and the integuments are strikingly similar in organization.

In 1876 WILLIAMSON (75) established the form genus *Lagenostoma*, distinguishing three species, *L. Lomaxii*, *L. physoides*, and *L. ovoides*. *L. Lomaxii*, the species selected in this work, has been fully described by OLIVER and SCOTT, who announced that the seed belonged to *Lyginodendron Oldhamium* (40), and later published a full account (41). *Lagenostoma physoides*, referred to by WILLIAMSON (76) as *Physostoma*, was studied by BUTTERWORTH (10), and finally worked out by PRANKERT (46). ARBER (2) described two additional species from impressions, as *L. Kidstoni* and *L. Sinclairii*. Unfortunately she said very little about the testal situation, and the account was more or less taxonomic. Other accounts are of minor importance, so far as the stony layer is concerned.

OLIVER and SCOTT (40, 41) described the structure of the seed of *Lagenostoma Lomaxii*, its pollination, and the morphology of the seed and the cupule. They showed the integumental situation and figured the true nature of the stony layer and surrounding tissues. In their accounts they described the canopy, palisade layer, chalaza, and vascular system. The canopy is the free part of the integument, and the rest of it is coalescent with the body of the seed for four-fifths of its height. The coalesced part consists of two kinds of elements, an outer palisade layer and an inner hypoderm. The superficial tangential section of the seed shows that the palisade layer is made up of cells arranged in longitudinal series, "running from pole to pole like circles of longitude." In transverse sections of the seed,

the palisade layer presents "the form of a continuous investment of radially elongated elements about 2μ in width, while near the apex it attains a much greater radial thickness (114μ)."

"In most specimens the palisade cells bear little pegs at the surface, not always inserted centrally, and having a height reaching $20-24\mu$ and occasionally even 30μ . Commonly these pegs are black and structureless, while occasionally the body of the peg appears to be inclosed in a little cap of membrane." DE HAAN (18) thinks that "perhaps they may be considered as the remainder of small cells on the outside of the palisade layer which have segregated mucilage."

The palisade layer in the small abortive seeds shows no differentiation at all, suggesting the situation in *Pinus* and *Juniperus*. OLIVER and SCOTT's findings regarding the palisade layer are most interesting and important. They say "such seeds are limited by a layer of small cubical cells which bear peglike papillae, apparently not yet separated by transverse walls. It may be supposed that at a later stage of development this layer divided tangentially into two layers, of which the thinner elongates to form the prismatic layer, whilst the outer survives in the form of pegs." While this case is not exactly analogous to the situation in *Pinus* and *Juniperus*, the development of the two layers in the integument in the latter seeds suggests an analogy.

In its whole organization the seed of *Lagenostoma Lomaxi* suggests the seed of a modern cycad. Below the level of separation, the testa (integument) and nucellus constitute one structure as in a cycad. The cupule, which was compared with the sarcotesta of the cycad ovule, will be discussed later. The palisade layer, which has been described as simple, cannot be taken as an index to show its antiquity because, even in the angiosperms, we have seeds with a simple stony layer as in *Sassafras* (figs. 81, 82, 83). The preservation of the palisade cells is very important, with the nuclei well shown. It is to be regretted only that no very young seeds of *Lagenostoma* have been collected, showing the structure of such a layer in a very young stage. It is likely that the stony layer in this seed developed as in *Sassafras*, in which it is differentiated at a very early stage as the one layer of cells marks itself out. The cells finally elongate, stretching without dividing. After attaining the

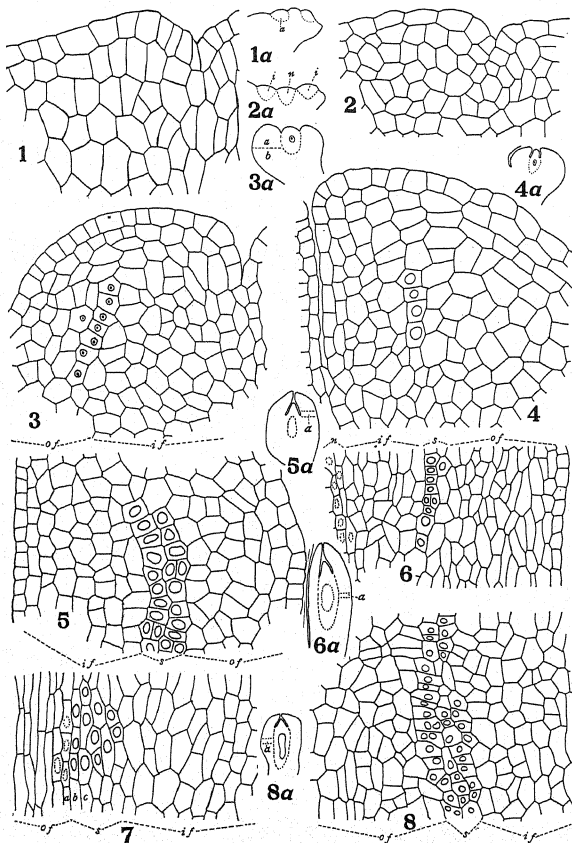
mature length, the stony layer starts to lignify, beginning with the walls in the outermost part of the cell, and finally becomes thickened throughout. Pitting in the stone cells is very common. Lignification starts from the micropylar end and gradually descends toward the chalazal region. In the seeds of *Lagenostoma* there is no indication that the cells in the palisade layer have become pitted.

The cupule of *Lagenostoma*, although it immediately surrounds the ovule, suggests the husk of *Hicoria*, in some species of which the husk is free at the micropylar end when ripe and dried. The general features of the husk of *Hicoria* and the adnate cupule of *Lagenostoma* suggest an analogy. The position of the stone also suggests a similarity. A study of these analogies will be undertaken in a future paper.

Cycadales

The integument of cycads is hypodermal in origin, and arises as an organ distinct from the nucellus. The elevation of the integument is the result of periclinal divisions of the hypodermal cells; and also there are anticlinal and radial divisions later. The intermediate stages (figs. 1-4) show the early development of the integument, after the first periclinal division of the integumental mother cell. These stages have developed somewhere between July 25 and August 8.

The integument (figs. 1, 2) shows that it is a homogeneous tissue, with cells generally hexagonal and uniform in shape. Fig. 1a shows a very young ovule before the megaspore mother cell is differentiated, but the archesporial region is already distinct. The meristematic region responsible for the elevation of the integument is shown in fig. 1, a detail of part of the integument in fig. 1a. The divisions are both periclinal and anticlinal, with some radial. The next stage of the developing integument (figs. 2, 2a) shows it outgrowing the top of the nucellus a little. The meristematic region of the integument is much broader here, and the tissue is homogeneous (fig. 2), with cells nearly uniform in shape and size. The development of the integument is coincident with the development of the archesporium. It begins to differentiate into two regions, an outer zone (the outer fleshy) and an inner zone (the inner fleshy). This differentiation starts as soon as the megaspore



FIGS. 1-8

mother cell is formed (figs. 3, 3a). The cells in both the outer and inner fleshy layers still show the traces of the old hexagonal type of cells, but the first indication of the demarcation line between the inner and outer fleshy layers is a row of somewhat uniform squarish cells. This row not only sets the outer and inner fleshy layers apart, but there is a difference also in the density of the cells in each region. The outer cells are less dense, and the nuclei are comparatively smaller, barely filling up the cells, and even the cytoplasmic radiations are not strongly developed. In the inner fleshy layer the nuclei of the cells are large, occupying nearly the whole cell, and the cytoplasmic radiations are much more pronounced, giving the cells a denser appearance. Cells below the level *a* (at *b* in fig. 3a) are of the type prevalent in the very young integument. The appearance of the squarish cells can be regarded as the limiting factor. The layer is formed by anticlinal division. The ovule represented in figs. 3 and 3a was collected about August 8 (56).

FIGS. 1-8.—Figs. 1-5, *Zamia floridana*: fig. 1a, diagram of median longitudinal section of very young ovule before megaspore mother cell is formed, showing meristematic tissue of developing integument (*a*), $\times 27$; fig. 1, details from *a* of fig. 1a, showing undifferentiated integument, $\times 502$; fig. 2a, diagram of median longitudinal section of ovule slightly older than fig. 1a, showing integument and nucellus, $\times 27$; fig. 2, integument in fig. 2a, showing homogeneous tissue, cells isodiametric, $\times 300$; fig. 3a, diagram of median longitudinal section of ovule when megaspore mother cell is already formed, $\times 12$; fig. 3, integument in fig. 3a, showing beginning of differentiation of outer fleshy and inner fleshy layers; all cells below level *a*, at *b*, fig. 3a, isodiametric, $\times 300$; fig. 4a, diagram of median longitudinal section of ovule older than fig. 3a, $\times 13$; fig. 4, integument of ovule in fig. 4a, showing early beginning of stony layer (*s*); cells of outer fleshy layer (*of*) near periphery beginning to orient, $\times 300$; fig. 5a, diagram of median longitudinal section of ovule collected August 29, with enlarged embryo sac, and before megaspore mother cell nucleus has divided, $\times 13$; fig. 5, portion of integument (*a*) from fig. 5a, showing two rows of cells of stony layer, with squarish or isodiametric cells, $\times 300$; fig. 6a, *Microcycas calocoma*: diagram of median longitudinal section of ovule in early free nuclear stage, $\times 13$; fig. 6, *M. calocoma*: integument from fig. 6a (*a*), showing very early differentiation of stony layer, stage slightly younger than shown in fig. 5, $\times 195$; fig. 7, *Ceratozamia mexicana*: portion of integument in median longitudinal section of ovule, showing further development of stony layer (*a*), inner row of cells belonging to outer fleshy layer (*of*), *b* and *c*, cells belonging to stony layer, $\times 195$; fig. 8a, *M. calocoma*: diagram of median longitudinal section of ovule in free nuclear stage, showing region (*a*) where details of fig. 8 were taken, $\times 8$; fig. 8, *M. calocoma*: portion of integument of ovule in fig. 8a, showing further development of stony layer, $\times 195$.

Figs. 7 and 8 were chosen from an ovule a little older than that shown in figs. 3 and 3a. It shows a much more developed nucellus, and still is in the megaspore mother cell stage. The integument has increased considerably in size. The comparative size of the nucellus and integument, with a detail of the tissues, is shown in figs. 4a and 4. The mode of growth of the integument checks the enlargement of the nucellus a great deal, and also causes the narrowing of the micropyle. This stage of the integument is little older than that in fig. 3, and shows the beginnings of the second row of somewhat squarish cells. In addition to such progress in the differentiation, fig. 4 also shows that the peripheral cells of the outer and inner fleshy layers begin to orient themselves, elongating in a longitudinal direction. The division of the megaspore mother cell into two cells, finally into four megaspores, and then the disintegration of the three and functioning of one, do not affect the integument at all in the development of its structure. Such divisions must have taken place rather rapidly.

The enlargement of the embryo sac begins about August 29, followed by growth of the surrounding tissues ("spongy tissue"). This further development of the embryo sac affects the tissues of the integument. The early beginnings of the stony layer seem to start after the megaspore has divided, and also when the spongy tissue shows considerable development. Figs. 5 and 5a show the origin of the stony layer; in fact, the squarish or isodiametric cells can be considered as the "stony mother cells." Their presence marks the outer from the inner fleshy layer. Divisions of these cells periclinally result in more squarish or isodiametric cells. This shows that the stony layer is of integumental origin, as far as the integument extends, and that the stony layer first originates from the innermost cells of the outer fleshy layer. This does not mean that the stony layer is composed wholly of cells of the outer fleshy layer. Further study of the stony layer will reveal that the inner fleshy layer also contributes to the formation of the stony layer of the old ovules.

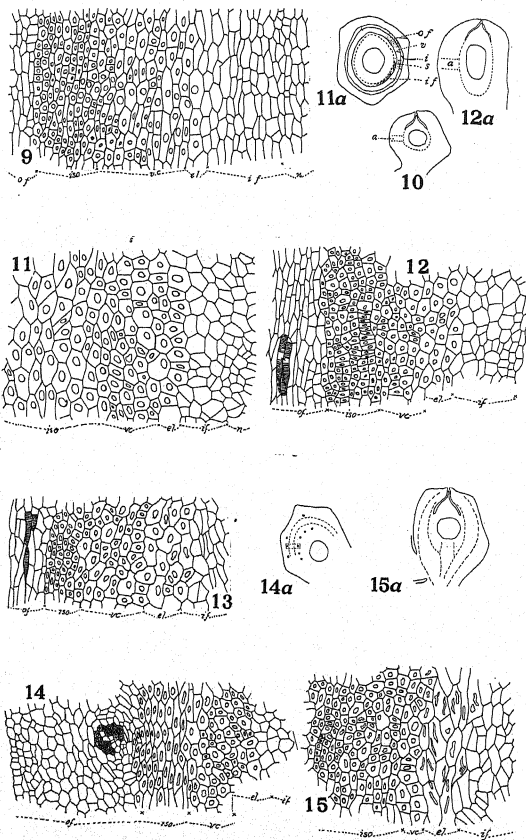
From the stages shown in figs. 6 and 6a the study of the stony layer will be based on different genera: *Microcycas*, *Ceratozamia*, *Dioon*, *Zamia*, *Cycas*, and *Stangeria*. A range of generic material will not only provide a range for study of variations in the develop-

ment and nature of the stony layer in these genera, but will give also an opportunity to connect the stages more critically, as it is difficult to obtain a complete series from one genus alone. With the exception of *Zamia floridana*, the stages in the different genera are interrupted. It will be found that the stages show an unbroken continuity with little modification, which will be noted in passing.

Microcycas calocoma (figs. 6, 6a) shows a striking resemblance to *Zamia* in the development of the isodiametric cells, the type first appearing in the life history of the stony layer. The cells in the outer and in the inner fleshy layer show a little more variation in shape than those of *Zamia*. In the former they are more or less elongated longitudinally. The cells in the inner fleshy layer begin to show a little distortion, some becoming somewhat compressed or flattened. As in *Zamia*, cells destined for the stony layer divide anticlinally. The stage of the embryo sac is like that of *Zamia* (figs. 5, 5a), collected August 5.

In the ovule of *Ceratozamia mexicana* (fig. 7), about the age of that of *Microcycas* (figs. 6, 6a), the outer fleshy layer becomes more active than the inner. Previous to the formation of the isodiametric cells of the stony layer the cells of the outer fleshy layer divide periclinally first, resulting in elongated cells (*b*, *c*). As in the two other genera, the formation of the isodiametric cells is by an anticlinal division. Fig. 7 (*a*, *b*) shows an intimate relation of the innermost row of cells of the outer fleshy layer and the young isodiametric cells of the stony layer. At this stage the inner fleshy layer has not yet differentiated.

Figs. 8 and 8a (*Microcycas calocoma*) show two rows of isodiametric cells already formed, and the cells in the outer fleshy layer near them beginning to show anticlinal division. An ovule of *Ceratozamia mexicana*, about 8×5 mm., and embryo sac with free nuclei in the stage of early wall formation show further stages of the development of the other three layers of the stony layer. It shows transitional stages of some of the isodiametric cells to the "vertical type," by failing to divide anticlinally and by slight elongation. It also shows transitions of the outermost layer of the inner fleshy layer to the so-called elongated longitudinal type of cells of the stony layer, by enlarging and expanding in a longitudinal direction.



FIGS. 9-15

Fig. 9 shows about eight layers of isodiametric cells and considerable thickness of the vertical cells, and the beginnings of the elongated longitudinal type of cells of the stony layer.

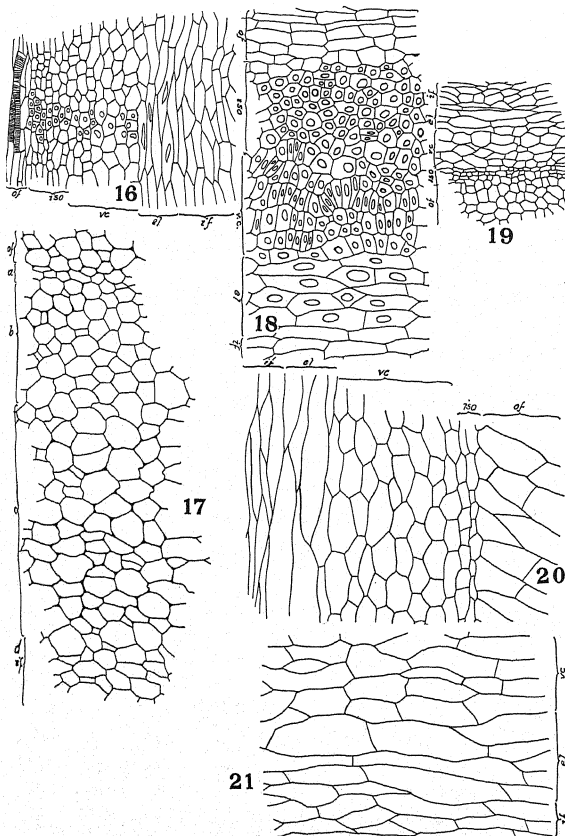
A *Zamia* ovule of the same age (fig. 10) shows the same phenomenon as regards the structure of the integument shown in fig. 8. The sharp definition of these three types of cells of the stony layer is shown in a cross-section of an ovule (figs. 11, 11a) of practically the same stage as fig. 9. The first outermost type, corresponding to the isodiametric cells (longitudinal section), are well differentiated from the vertical cells by their shape. The elongated longitudinal type (of the longitudinal section) is differentiated from the vertical cells by the size and by having in most cells round corners rather than sharp and angular ends. The inner fleshy layer can be distinguished from the elongated longitudinal type by having sharp angles and a rather squarish shape. There is only one layer of the somewhat roundish type of cell (elongated longitudinal in longitudinal section). The growth of the ovule is accompanied by the differentiation of the three types of cells of the stony layer. The stony layer at first grows in thickness by periclinal divisions and by the sacrifice of some other cells. Figs. 12 and 12a (*Microcycas calocoma*) show that the

FIGS. 9-15.—fig. 9, *Ceratosamia mexicana*: portion of median longitudinal section of integument, showing eight layers of isodiametric cells (*iso*), vertical cells (*vc*), and beginning of elongated longitudinal type of cells of stony layer (*el*), $\times 166$; fig. 10, *Zamia floridana*: diagram of median longitudinal section of ovule repeating situation in fig. 8a, $\times 6$; fig. 11a, *Microcycas calocoma*: diagram of transverse section of ovule, showing different layers; outer fleshy layer (*of*), isodiametric cells (*iso*), vertical cells (*v*), early beginning of elongated longitudinal cells (*el*), inner fleshy layer (*if*), $\times 6$; fig. 11, *M. calocoma*: portion of integument from fig. 11a, showing stony and fleshy layers, $\times 195$; fig. 12a, *M. calocoma*: diagram of median longitudinal section of ovule, showing region (*a*) where details in fig. 12 were taken, $\times 6$; fig. 12, *M. calocoma*: details of *a* in fig. 12a, showing thick region of isodiametric cells, vertical cells, and elongated longitudinal cells contributed by inner fleshy layer (*if*), $\times 195$; fig. 13, *Z. floridana*: portion of integument from median longitudinal section of ovule, showing fleshy layers, and comparatively thin stony layer, $\times 166$; fig. 14a, *Z. floridana*: diagram of transverse section of ovule, showing region where details of fig. 14 were taken, $\times 6$; fig. 14, *Z. floridana*: details of *a* from fig. 14a, showing vascular bundle in outer fleshy layer, different layers of stony layer, and portion of inner fleshy layer, $\times 166$; fig. 15a, *Z. floridana*: diagram of median longitudinal section of ovule, showing region where details in fig. 15 were taken, $\times 6$; fig. 15, *Z. floridana*: details of *a* from fig. 15a, showing different layers of stony layer, with inner layer showing extreme elongation of cells, $\times 166$.

inner fleshy layer at this stage has contributed two or three layers of cells to the elongated longitudinal type of cells of the stony layer. It is very well differentiated by its size, and also by the form of the cells. It is very evident from fig. 12 that the vertical cells of the stony layer are the result of failure of the isodiametric cells to divide anticlinally. Fig. 13 (*Zamia floridana*) shows an ovule in the early stage of wall formation of the nuclei in the embryo sac. It gives an idea of the comparatively simple stony layer, and also the actual thickness of the whole stony layer. The isodiametric cells are comparatively thin, of about five rows of cells, the vertical cells of about the same number, and three rows of the elongated longitudinal type. The figure also presents a picture of the later stage of transformation of the vertical and elongated longitudinal type of cells of the stony layer. The cells are becoming irregular and wavy in form previous to elongation, a phenomenon due to the growing ovule. An ovule of the same age, in cross-section (figs. 14, 14a), gives an idea of the great thickness of the outer fleshy layer and the comparatively thin inner fleshy layer, which is now only about four layers thick. The outermost cells of the stony layer (isodiametric in the longitudinal section) are elongated longitudinally with sharp ends; the vertical cells show irregularities in form from an orbicular type to the stretched longitudinal cells. The round corners, due to intercellular spaces, also are characteristic. The third type of cells of the innermost part of the stone shows an intimate association with the inner fleshy layer, not only in the resemblance of cell form, but also in the displacement. The nuclei of the elongated longitudinal type of cells are pointed (figs. 15, 16), and even others show stages of disorganization. This state of disorganization is valuable in the study of the lignification of these cells. While nuclei may be found occasionally in the isodiametric cells in the stony layer, they generally disappear and are entirely absent in the well lignified cells. The further development of the stony layer is accompanied by continuous elongation of its longitudinal cells. They often elongate enormously to form structures like a fiber. In *Cycas revoluta* the differentiation and development of stony layer until the formation of the archegonium are practically the same as in the other genera described, the only difference being

in the number of layers composing the stony layer. Because of the ovoid form of the ovule, there are more layers of isodiametric and vertical cells than are generally found in *Zamia*.

While *Zamia*, *Ceratozamia*, *Microcycas*, *Stangeria*, and *Cycas* offer examples of a rather simple stony layer, possessing three types of cells, *Dioon edule* shows the most complex one in this stage. The development of the four types of cells which constitute the stony layer and the reason for such complexity are illustrated by fig. 18. The development of the isodiametric cells is identical with that in other genera, but the origin of the vertical and transverse type of cells is different. The origin of the elongated longitudinal type, the fourth type, is similar to that in the other genera. They are products of the inner fleshy layer, and both show intimate connection with each other. The inner fleshy layer is differentiated from the elongated longitudinal cells of the stony layer by its even orientation, and by having perpendicular rather than slanting walls. The cells are stretched and flattened somewhat, due to the growth of the ovule. The flattening of these cells is due to the centrifugal and centripetal forces acting upon them on both sides. The further stretching of the elongated longitudinal cells of the stony layer and the outermost ones of the inner fleshy starts from the micropylar end and gradually descends to the chalazal region. The isodiametric cells show great resemblance to the innermost cells of the outer fleshy layer. Of course the peripheral cells of the outer fleshy layer have changed in shape and have assumed a parallel arrangement. The second type of cells (the vertical) must have originated from the isodiametric cells, but on account of the pulling effect apically, due to the growing integument, they tend to stretch and assume a more or less twisted shape. In the mature ovule these cells assume, more or less, the form of an S, which is not so conspicuous when the ovule is ripe. The third type of cells does not stretch as much as the second type. The cells divide periclinally to form the so-called transverse type. When the ovule is small, as in *Zamia*, the structure of the stony layer is not so complex (fig. 19). In the latter part of the life history of an ovule of *Zamia*, about 22×11 mm., the isodiametric cells of the stony layer are reduced to about two layers only, making a reduction of five to two. Some of these

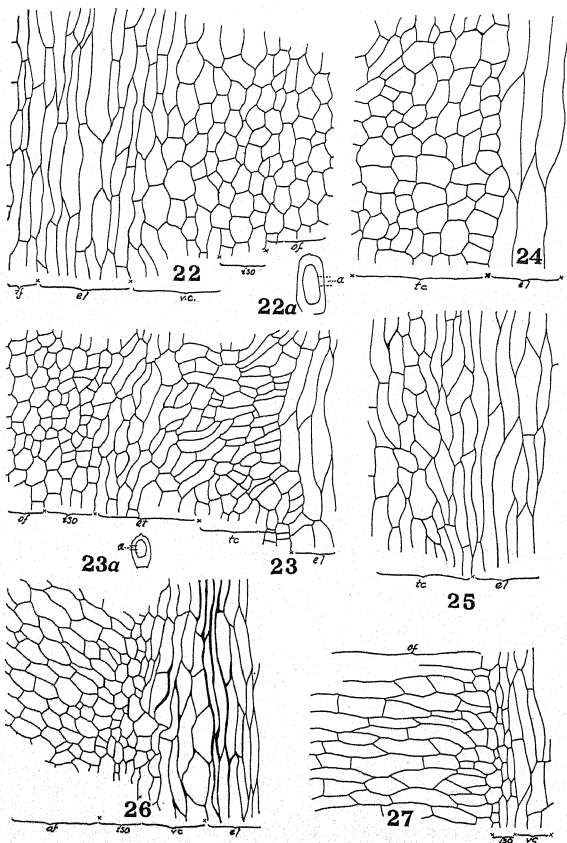


FIGS. 16-21

cells are being used in the formation of the vertical type of cells (fig. 20). The sequence of events in the development of the different layers of the stony layer is interesting. The last in the life history lignifies first, and the first to appear (the outermost) lignifies last.

During lignification the cells of the stony layer show many marked changes. After the three distinct layers have been differentiated, the next step is the transition of one kind of cell to another. Conspicuous in this is the further transformation of the isodiametric cells of the stony layer to the vertical type, by the cessation of cell division and enlargement of the cells, followed by elongation. When this stage is reached, the vertical cells resemble, more or less, the elongated longitudinal cells (figs. 16, 17, 19, 20, 21). In fig. 17 one sees the reduction of the number of isodiametric cells reaching across the layer, from about five to seven, in young stages (fig. 9), to about two layers in this stage. The other transformed isodiametric cells, after enlargement (*b*), have a more or less uniform shape, and are angular. There are distinct intercellular spaces. Cells from the outer fleshy layer to *b* are thin and parenchymatous, but below the level of *d* the walls are beginning to thicken, while at the end of level *c* no thickening is taking place. Walls are double and thickest at level *c*. At this level the tissue is further characterized by the presence of an enormous number of tannin cells, especially in the elongated longitudinal type of cells. The thickening and distinct doubling of the innermost walls, adjacent to the inner fleshy layer, are signs also of approaching lignification. The walls with rounded corners of the vertical and elongated longitudinal cells of the stony layer (in cross-section) are differentiated from those

FIGS. 16-21.—fig. 16, *Ceratosamia mexicana*: portion of integument in median longitudinal section of ovule, showing transitional stages of two outer layers of stony layer, and extremely elongated longitudinal cells of stony layer, $\times 166$; fig. 17, *C. mexicana*: portion of integument in transverse section of ovule, showing cells of stony layer (*a*, *b*, *c*), $\times 166$; fig. 18, *Dioon edule*: portion of integument in median longitudinal section of ovule, showing three types of cells of stony layer; complex (*s*) cells not yet formed, $\times 195$; fig. 19, *Zamia pumila*: portion of integument in median longitudinal section of ovule, showing very simple stony layer and its thickness, $\times 57$; fig. 20, *Z. floridana*: portion of integument in median longitudinal section of ovule, showing vertical cells beginning to elongate, and very extreme elongated longitudinal cells; outer fleshy layer showing elongated transverse cells, $\times 195$; fig. 21, *C. mexicana*: elongation of vertical cells of stony layer, $\times 166$.



FIGS. 22-27

of the isodiametric type, which are angular and penta-hexagonal. The third stage of the development of the stony layer is characterized by the extensive stretching of the vertical cells (fig. 21). They not only stretch like the elongated longitudinal cells, but also show a thickening of the walls. Stretching is most developed in an ovule when the embryo sac has enlarged a great deal previous to fertilization of the egg. Fig. 22 shows the elongated longitudinal cells very much stretched, and the vertical cells adjacent to them also elongating. In an old ovule of the same age (fig. 22a) the isodiametric cells have undergone changes in form, except in one or two layers that are adjacent to the outer fleshy layer. The isodiametric cells of these two layers show all stages of transition in form and even in thickness of walls from isodiametric to cells with more or less roundish walls, and to elongated vertical cells. The walls become thicker within until they are distinctly double and are thick in the elongated longitudinal type. An ovule of the same size and age of *Microcycas calocoma* shows the same behavior of the developing stony layer.

The most complex stony layer reported by several investigators, so far as the nature of these layers within are concerned, is not really very complex, if studied in series and at different stages. A *Dioon edule* ovule 8×3 mm. shows the three types of layers distinctly (fig. 18). The first outermost cells are the isodiametric, and the middle layer is composed of vertical cells, which originated from the isodiametric cells by anticlinal divisions. Fig. 18 shows the

FIGS. 22a-27.—fig. 22a, *C. mexicana*: diagram of median longitudinal section of ovule, showing region where details in fig. 22 were taken, ×50; fig. 22, *C. mexicana*: details from a, fig. 22a, showing transitional stages in elongation of cells of stony layer, and also change in wall structure of isodiametric cells, ×166; fig. 23a, *D. edule*: diagram of median longitudinal section of ovule, showing region where details in fig. 23 were taken, ×50; fig. 23, *D. edule*: details from a, fig. 23, showing vertical cells being transformed into S-shaped cells, formation of transverse cells, and elongated longitudinal cells of stony layer, ×195; fig. 24, *D. edule*: transverse cells and elongated longitudinal cells of stony layer, ×195; fig. 25, *D. edule*: integument in median longitudinal section, showing change of transverse cells to elongated longitudinal cells, ×195; fig. 26, *Z. floridana*: portion of integument of median longitudinal section of ovule, near micropylar end, showing transverse stretching of cells of outer fleshy layer, with portion of stony layer, ×166; fig. 27, *Z. floridana*: portion of integument from ovule older than in fig. 26, showing extreme elongation of outer fleshy layer cells, ×166.

beginnings of the twisting of the vertical cells. The innermost cells of the stony layer are of the elongated longitudinal type. A little older stage (figs. 23, 23a), when the ovule is 13×8 mm., shows more markedly the displacement of the cells of the stony layer. Even the isodiametric cells are being stretched somewhat in the apical direction. The vertical cells have stretched a great deal and are beginning to show the *S* form. The stretching of the vertical cells is followed by enlargement, thus eliminating the more pronounced *S* form. Fig. 24, instead of having such *S* form cells of the vertical type, shows irregular forms, with more or less rounded corners. Even the number of layers of the isodiametric cells has been reduced, a reduction very common in all genera of cycads. Figs. 24 and 25 show stages in the developing cells belonging to the vertical type innermost and adjacent to the elongated longitudinal cells. While fig. 24 is from a much older ovule than that in fig. 25, the transition is very marked. In this figure the squarish cells found in the innermost region of the vertical cells are still preserved. In a mature ovule this is characteristic, especially near the middle portion; but somewhere near the apex, where stretching is most evident, these squarish cells also show stretching (fig. 25), which continues until the growth of the ovule ceases. In a mature ovule these squarish cells have undergone enormous stretching, often resembling the elongated longitudinal type, except that they are comparatively shorter. This is more complex in *Dioon edule* than in *Zamia floridana* (fig. 20), but the final product and nature of the stony layer are almost identical in all genera. While in *Zamia floridana* the transition of one cell to the other is simple, in *Dioon edule* it is quite complex. The elongated, longitudinal, *S*-shaped cells and the transverse (squarish) cells of the vertical cells are transitional stages and not final.

Following the thickening of the walls is the doubling. The chemical phenomena taking place in this doubling are not known, but indications of their nature throw light upon the problem of lignification. The nature of the walls of the stone cells in a mature ovule is shown in pl. figs. 4, 4a. For the mature stages the apical and chalazal regions of the stone cells give the best material, but for very young stages of lignification one has to search in the cells

near the middle part of the ovule. Whether the nucleus and tannin in cells play a part in the lignification the writer is not able to ascertain; but observations show that these cell contents have something to do, as in the case of protoplasmic inclusions these materials follow practically the same life history in the formation of pits. Tannin persists in the life history even when the cells are already lignified and hard. The nucleus in some cells persists, but in such cases it does not take part in the lignification; but disorganized nuclei probably do take part. In cells where no nucleus or tannin takes part, the protoplasm and the vacuolar contents are important. In this case, the protoplasm seems to be made of very fine reticula with very minute granules. Under the high power dry lens these inclusions seem to appear homogeneous; but examining them under an immersion lens, the homogeneous structure looks very much more reticular and granular. Large and small granules are distinguished. In young stages the nucleus is surrounded by radiating cytoplasm with conspicuous vacuoles. The cell sap in these vacuoles probably undergoes some chemical changes as the cells get older. The cell inclusions are dense near the walls, and light and vacuolated near the center of the cell. This dense portion contains a quantity of the large granules, which orient themselves and appear as a heavy line under the low power lens. In mature stages these granules produce the double character of the walls (pl. figs. 1-3). During the development of the stone cells these granular materials first found in the cytoplasmic strands are attracted to the walls, and the successive piling on the walls gives the dense condition. Following the orientation of these granules and other material upon the walls comes vacuolation, which also starts near the walls and gradually moves to the center (pl. fig. 3). Vacuolation is often spontaneous, and is responsible for the pitting in these stone cells. The presence of several of these slits gives an appearance of a branching pore. Vacuolation is due to the aggregation of these granules and reticular fibers (pl. fig. 4a).

Pitting can be formed without the participation of the nucleus or tannin. When the nucleus takes part, the first stage noted is the rupture of the nuclear membrane, which allows the nuclear contents to be dispersed in the cell. This mass plus the cytoplasmic material

now diffused throughout gives a denser look to the cells. Most of the granular matters are piled up on the walls and the rest dispersed all over, and are often connected by threadlike structures. This starts actively at the apical region of the integument, the elongated longitudinal cells being the first to react, then the vertical cells. Simultaneously the basal cells also lignify, but instead of being elongated longitudinally and stretched, they are more or less elongated horizontally and interlaced with one another. Somewhat isodiametric cells are found, especially near the regions of the vascular bundles. Lignification seems to proceed faster at the chalazal region than at the apical portion; and while it is going on in the stony layer, some cells of the inner fleshy layer most intimately connected with the stony layer also lignify. This is especially true near the chalazal end. This lignification of some of the inner fleshy cells explains why in the ripe seeds the basal end of the stone is often adnate to the papery inner fleshy layer.

In the ripe seeds there are in general two layers of cells of the stony layer. While in the developing ovule three or four layers are distinguished, the final result is two-layered, the two layers of cells differing not only in form and size, but also in the nature of the walls, especially the secondary thick walls. The cells of the outermost layer adjacent to the outer fleshy layer are smaller, isodiametric, with more or less rounded lumina and rounded corners (pl. fig. 5).

Pitting is universal in the developing stone cells. It disappears as a general rule when the cells have become hard and lignified, but sometimes we find isolated cells with pits. The tissues around the pits during early lignification show a homogeneous structure with no signs of slits or pores. Later this becomes somewhat vacuolated by enlargement of these pores. This vacuolation continues (vestiges of this seen in pl. fig. 6) and forms pores. At the same time the pits at the center coalesce, and those near the periphery of the walls elongate. When two pits happen to be in line on opposite sides, and vacuolation and elongation in longitudinal direction continue, they form the "pores," a kind of canal which establishes connection between the lumen of one cell and the other. In the stone cells these pores are much lighter and less dense.

Between these are dense portions which form the thick coating of the walls, which is originally of the tissue surrounding the pits. Striations in this thickening are very evident, even in stages before the final orientation of the material to the walls. The cells from the outer layer differ not only in the form (isodiametric), but also in the size of the lumina. There are again more pores, and the structure of the walls is quite different. The thickening is made up of superposed layers. The nucleus sometimes persists, and if it does it is colorless and the cytoplasm surrounding is disorganized (pl. fig. 5). Cells near the inner fleshy layer are elongated, and in *Ceratosamia* many are still filled with tannin (pl. figs. 6, 8). While generally the pits disappear at maturity, they sometimes persist. These cells afford a good illustration of the development of the walls after pitting (pl. fig. 6). This figure illustrates also stages from homogeneity until the same tissue becomes striated. Cells near the inner fleshy layer, belonging to the elongated longitudinal and vertical type of the stony layer, are quite simple in organization. There are fewer pores, but the secondary walls are quite complex. They are practically double-layered; the portion adjacent to the primary wall has the vertical striations, while the layer toward the lumen has parallel striations. The lines, instead of being more or less straight, are slightly wavy (pl. fig. 7). Cells with tannin (pl. fig. 8) have more or less rounded corners and show the same double layered secondary wall. The pores are also filled with tannin and very often the ends of the papillae are enlarged. While the opposite papillae show intimate connection, no protoplasmic strands could be found connecting the two papillae. One might be able to see strands if the material were fixed and stained for protoplasmic connections.

The stony layer in the different genera of cycads studied shows variation in thickness, shape, and also in the structure of the layers which constitute it. In general the stony layer is simple, except in *Cycas revoluta* and *Dioon edule*. The stony layer of *Ceratosamia mexicana* can be regarded as typical of the Cycadaceae, with *Cycas revoluta* and *Dioon edule* as exceptions. In *Zamia floridana* the stony layer is two-layered, both in transverse and in longitudinal section. The outer layer is composed of elongated longitudinal cells in transverse section, the cells varying in size, being smallest

in the outermost part, and gradually increasing in size as they approach the inner layer. The inner layer is made up of isodiametric cells of varying lengths, the smallest being near the inner fleshy layer. In longitudinal section the stony layer shows little variation. The outer layer is made up of isodiametric cells and the inner layers of elongated longitudinal cells, which diminish in size as they reach the inner fleshy layer, but are comparatively longer. The stony layer in *Microcycas calocoma* is practically the replica of that of *Zamia floridana*, both in arrangement of cells and in structure, but the walls are not as thick as those of *Zamia floridana*. The region of isodiametric cells (belonging to the outer layer in longitudinal section) is quite extensive, often occupying more than the whole width of the stony layer. The study of *Cycas revoluta* shows that there are two layers, but in addition the stony layer is quite complex, nearly comparable with that of *Dioon edule*, but not as complex. While in the other genera studied the two layers of the stony layer are very distinct in their orientation and form of cells, *Cycas revoluta* shows some variation worth noting. The stony layer is thicker than in *Ceratozamia mexicana*, *Zamia floridana*, and *Microcycas calocoma*. In a transverse section the outer region is made up of small isodiametric cells on the outside, gradually increasing in size as they reach the middle region of the stony layer. While this is true, we have a mixture of elongated longitudinal cells sandwiched in between the isodiametric cells. This gives rather complex structure, but not as complex as that of *Dioon edule*. In all genera studied, in the young stages before lignification there are three rather distinct layers, the isodiametric type on the outside, the vertical in the middle, and the elongated longitudinal on the inside. The vertical type due to the growing ovule disappears at maturity, being changed to the elongated longitudinal type. In *Dioon edule* there are four layers in the young stages before lignification, but finally only two or three, with the third one rather arbitrary. The third layer often remains wavy, a reminder of the old S type of cells. The outer layer is made up of isodiametric cells which increase in size as they approach the arbitrary third layer. The elongated transverse cells, common in young stages, disappear at maturity. The inner layer of the stony layer is made up of

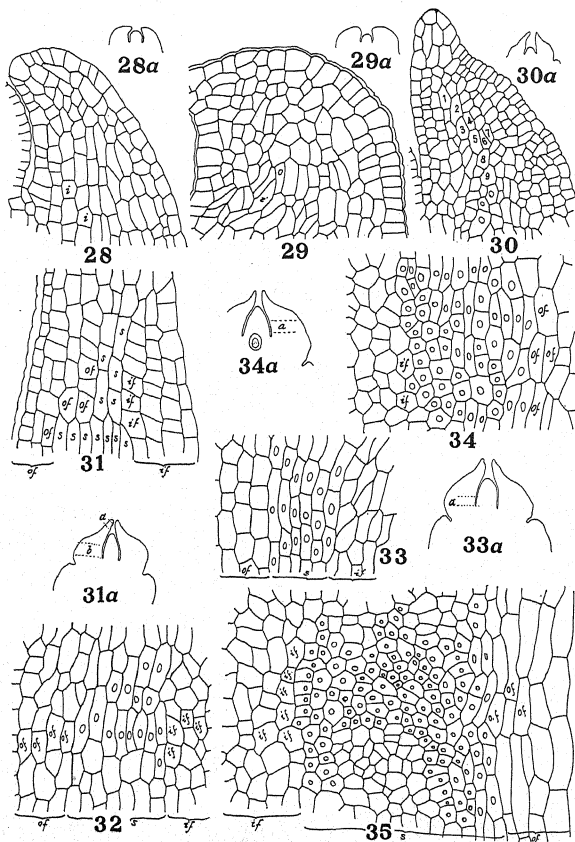
elongated longitudinal cells, increasing in length as they approach the inner fleshy layer. The cells in the chalazal region are isodiametric.

Ginkgoales

A very young integument of *Ginkgo* before the megaspore mother cell was formed (figs. 28, 28a) shows that the tissue is homogeneous. This is a replica of conditions found in cycads. The material was collected very early in the spring.

Although the tissue is homogeneous, the form of the cells suggests a little difference. The young cells give an impression of the isodiametric form (fig. 28, *i*), but on growth and development of the integument these cells become stretched somewhat to the elongated longitudinal type. This shape is due to the growing tip of the integument. Soon after the integument has outgrown the nucellus, and the megaspore mother cell becomes recognizable, a change takes place in the integument, differentiating the outer and inner fleshy layers. Figs. 29 and 29a show a very early differentiation of these layers. The cells in the outer fleshy layer still show the elongated longitudinal form, while the inner fleshy cells are becoming somewhat twisted. The outermost cells of the outer fleshy layer adjacent to the epidermis divide antichinally and orient themselves with the epidermis. The tips of the integument grow toward the nucellus first, but later they become erect, giving a saddle-like form to the apical portion of the integument (fig. 30a). The development of the megaspore mother cell is coincident with the development of the integument.

Fig. 30 is an older integument, when the megaspore mother cell has begun to enlarge and vacuolation has started. A row of cells is formed which marks the demarcation of the outer and inner fleshy layers. From analysis this row of cells belongs to the outer fleshy layer. The origin of the stony layer is dependent upon the orientation of the cells belonging to the fleshy layers. From division of cells 1 and 2 (fig. 30), the first layer of cells of the stony layer is developed. Cells 3, 4, 5, 6, and 7 show that periclinal division has taken place. In all cases the cuticle of the epidermal cells of the integument is very thick (fig. 31). While the illustrations to follow are almost always drawn at the level a little above the base of the



FIGS. 28-35

nucellus, the figures of the apex of the integument (figs. 31a (a) 31) illustrate the method of formation of the stone cells and the origin of the first stone cells. At the very apex (fig. 31) the two layers of the stone cells show that the cells are differentiated from the outer and inner fleshy layers. They are elongated longitudinally, while the outer and inner fleshy cells are more or less squarish; the walls of the inner fleshy cells being formed by radial division, while those of the outer are formed by anticlinal division. Somewhat lower there are several layers of stone cells which show their origin from the outer fleshy layer rather than from the inner. While in cycads the first type cells of the stony layer are isodiametric, in *Ginkgo* they are composed of the elongated longitudinal type of cells. Fig. 32 was taken at level *b* (fig. 31a), and shows uniformly elongated longitudinal stone cells. They show intimate relation with the cells of the outer fleshy layer, and the inner fleshy cells show still the radially arranged walls. The tissue of the integument behaves practically the same as in fig. 32, when the megaspore mother cell has enlarged a great deal, and vacuolation has become more pronounced.

As the integument grows both in thickness and in height, the inner

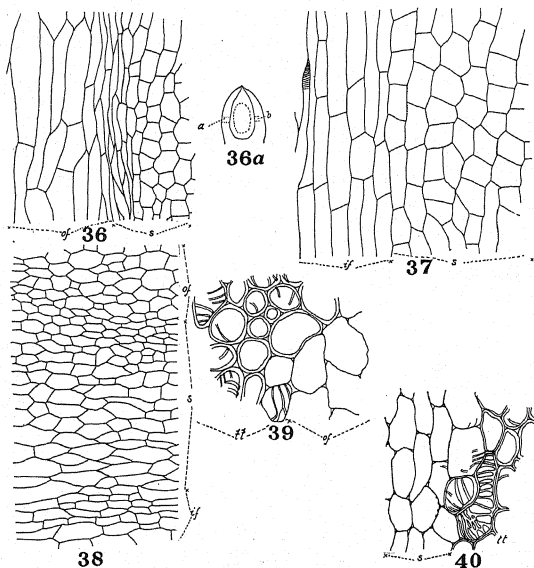
FIGS. 28a-35.—*Ginkgo biloba*: fig. 28a, diagram of median longitudinal section of very young ovule before megaspore mother cell is formed, $\times 23$; fig. 28, integument shown in fig. 28a, with homogeneous, undifferentiated tissue, $\times 195$; fig. 29a, diagram of median longitudinal section of ovule rather older than in fig. 28a, $\times 23$; fig. 29, integument in fig. 29a, showing very early differentiation of outer (*o*) and inner (*i*) fleshy layers, $\times 195$; fig. 30a, diagram of median longitudinal section of ovule in early differentiation of megaspore mother cell, $\times 13$; fig. 30, integument of fig. 30a, showing further differentiation of outer (1, 2, 3, etc.) and inner fleshy layers, $\times 166$; fig. 31a, diagram of median longitudinal section of ovule where details in figs. 31, 32 were taken, $\times 12$; fig. 31, details of *a*, fig. 31a, showing early differentiation of stony layer (*s*) and fleshy layers on both sides, $\times 166$; fig. 32, details of *b*, fig. 31a, showing first type of cells of stony layer (*st*), isodiametric cells belated, $\times 195$; fig. 33a, diagram of median longitudinal section of ovule; megaspore mother cell increased in size, and 3-4 rows of archesporial cells, $\times 12$; fig. 33, details of *a*, fig. 33a, showing intimate connection between juvenile stony layer and outer fleshy layer, and longitudinal stretching of inner fleshy layer cells, $\times 195$; fig. 34a, diagram of median longitudinal section of ovule in early free nuclear stage, $\times 6$; fig. 34, details of *a*, fig. 34a, showing formation of isodiametric cells by periclinal divisions of elongated longitudinal cells, $\times 300$; fig. 35, portion of integument in median longitudinal section of ovule much older than in 33a, showing thick stony layer, $\times 166$.

fleshy layer cells are subjected to stretching (fig. 33). Fig. 33 was taken from level *a* of fig. 33a. The stone cells, however, resist such a pull. The intimate connection of the stony layer and the outer fleshy layer is best illustrated in fig. 33, the outer fleshy cells having quite uniform rectangular cells, elongated longitudinally and somewhat enlarged. At one time in the division of the megaspore mother cell another type of cell appears in the stony layer. At the time of the second division, which forms four free nuclei in the embryo sac, there is a further stage in the development of the stone cells. In cycads the isodiametric cells are first in appearance, but in *Ginkgo* they are belated and appear in a different region. In cycads the isodiametric cells are localized at the outermost region of the stony layer adjacent to the outer fleshy layer; in *Ginkgo* they originate from the elongated longitudinal cells innermost and adjacent to the inner fleshy layer (figs. 34, 34a). The behavior of the isodiametric cells is probably due to the absence of a bundle in the outer fleshy layer.

The elongated longitudinal cells of the stony layer are formed by periclinal division, and the isodiametric cells by anticlinal division of the elongated longitudinal cells (fig. 34), starting from the inside and working outward. The innermost cells of the stony layer are differentiated from the inner fleshy cells, not only in their form, but also in the sharpness of the angles. In fig. 35 there are distinctly two layers of cells, a very thick layer composed of isodiametric cells, and one row of elongated longitudinal cells which failed to divide anticlinally. This behavior of the outermost row of cells of the stony layer shows again the intimate relation of the outer fleshy cells and the outermost stone cells. The cells resemble in form those of the outer fleshy layer, although the latter are longer and larger.

As the ovule grows and the archegonium is formed, the parenchymatous cells of the outer fleshy layer swell enormously. This enlargement tends to crush and compress some of its cells adjacent to the stony layer, but the stone cells suffer little compression. Simultaneous with this enlargement and elongation of the cells of the outer fleshy layer, the cells of the inner fleshy layer also elongate (fig. 37). The isodiametric cells also enlarge and elongate. Fig. 37

shows some isodiametric cells beginning to elongate, thereby losing the isodiametric form. While all the innermost cells of the stony layer elongate, some retain their form, and this explains why isodiametric cells are found in the innermost layer in the old seeds.



FIGS. 36a-40.—*Ginkgo biloba*: fig. 36a, diagram of median longitudinal section of ovule, showing region where details in figs. 36, 37 were taken, $\times 2.50$; fig. 36, details of a, fig. 36a, showing elongated longitudinal cells of outer fleshy layer (af) and portion of stony layer (s), $\times 166$; fig. 37, details from b, fig. 36a, showing elongated longitudinal cells of inner fleshy layer (if) and portion of stony layer (s), $\times 166$; fig. 38, transverse section of ovule 20-21 mm. in diameter, showing cells of outer fleshy, stony, and inner fleshy layers, $\times 166$; fig. 39, transverse section of ovule near base, showing transfusion tissue and cells of outer fleshy layer, $\times 195$; fig. 40, transverse section of ovule, taken near middle level, showing transfusion cells (tt) and stone cells before lignification; transfusion cells in this ovule disappear near micropylar end, $\times 195$.

Before presenting the subject of lignification, we may consider what possible relation the food contents of the three layers of the integument hold to the process. The cells of the outer fleshy layer are very rich in tannin, which is localized in *Ginkgo*, but in cycads is found in the outer fleshy layer, the inner fleshy layer, and even in the stony layer. It cannot be inferred that tannin forms a vital part in the lignification of cells, because there are cycads, like *Stangeria*, with no tannin at all in the integument, and yet lignification proceeds unhampered. The outer fleshy layer of *Ginkgo* is also well supplied with oxalate crystals, as in cycads, and with an abundance of starch and chlorophyll. Tests show no starch or chlorophyll in the stony layer or in the inner fleshy layer; but both are abundant in the nucellus and the nucellar region. The tissue surrounding the pits gives no reaction to lignin tests, but secondary walls begin to show a slight response. Cells of the stony layer react plainly to the lignin test.

The first glimpse of lignification is practically cycadean in nature, with little variation as lignification progresses. The behavior of the cytoplasmic material is conspicuous (pl. fig. 9). A cross-section of the ovule, under a high power dry lens, shows streaks of cytoplasmic material arranged as if they were cross-walls in cells. Further examination shows that the granular matter of the cytoplasm arranges itself across the cell. The nucleus does not disintegrate yet, and seems to play no part at all (pl. fig. 9). In this stage the walls are not yet thickened, but thickening is the next step. Lignification starts from the inside and gradually proceeds outward, a cycadean behavior. Lignification, however, starts faster from the chalazal end than from the apical. The rapidity of lignification at the chalazal end is probably related somewhat to the presence of transfusion tissue at the base of the ovule surrounding the bundles. The reticulations of the cells of the transfusion tissue (figs. 39, 40) resemble that of scalariform tracheids. One may be confused by these cells at the base, but the stone cells can always be distinguished from the transfusion cells by the shape. Much higher from the base of the ovule, the transfusion cells disappear, and leave the stone cells differentiated. Pitting takes place as in cycads (pl. fig. 10), and, as in cycads, the pits disorganize and fuse

together, leaving a lumen at the center of the cell, and thick walls at the periphery. Pores are produced by pits, and forking of these pores is quite universal in *Ginkgo*.

In a cross-section the stony layer presents two layers as regards form of cells; but if the nature of these cells be taken into consideration, there are three layers. The outermost is thinnest, and the cells, although slightly more elongated than the isodiametric cells, are practically like them. The innermost are elongated longitudinally. In longitudinal sections (pl. fig. 11) there are three distinct layers in the stony layer. The outermost consists of one row of elongated longitudinal cells. Then come several rows of isodiametric cells, and in the interior layer the cells are elongated longitudinally. Often isodiametric cells are found sandwiched in between these elongated longitudinal cells, the reason for which has been presented. The innermost layer of the outer fleshy layer does not lignify, and this is why the outer fleshy covering peels off very easily from the stony seed when the latter is ripe. The outermost cells of the inner fleshy layer, on the other hand, have undergone lignification slightly and adhered to the stony layer, especially in the basal half of the ovule.

With the exception of free forking in the isodiametric cells, the stone cells are practically cycadean in nature. While in *Ceratozamia* the nuclei of the stone cells seem to disappear in old age, in *Ginkgo* they remain undisturbed until the stone cells are completely sclerified. The nuclear form very often gives the center of the lumen a circular form (pl. fig. 12). In the elongated cells the lumen is narrow and well stretched. Small pits are found scattered in the thick secondary walls in both types, but more so in the isodiametric cells.

Coniferales

The large number of genera and species of Coniferales show variations in the structure of the female flower, ranging from ovules exposed and devoid of external covering to ovules enveloped by a fleshy covering. While the covering is permanent at maturity in the last case, the ovules are exposed in the early stages, a situation persisting in ovules without the fleshy covering. Studies in this order are based on *Pinus*, *Juniperus*, and *Torreya*, one with undevel-

oped fleshy covering and exposed, and the others with persistent, fleshy, and conspicuous envelopes covering the seeds. *Pinus Banksiana*, *P. excelsa*, *P. Laricio*, *Juniperus virginiana*, and *Torreya taxifolia* were investigated. Other species of *Pinus* and *Juniperus* were examined for comparison only. These species present a simple, comparatively narrow integument, which is favorable for a study of the origin of the stony and fleshy layers.

PINUS

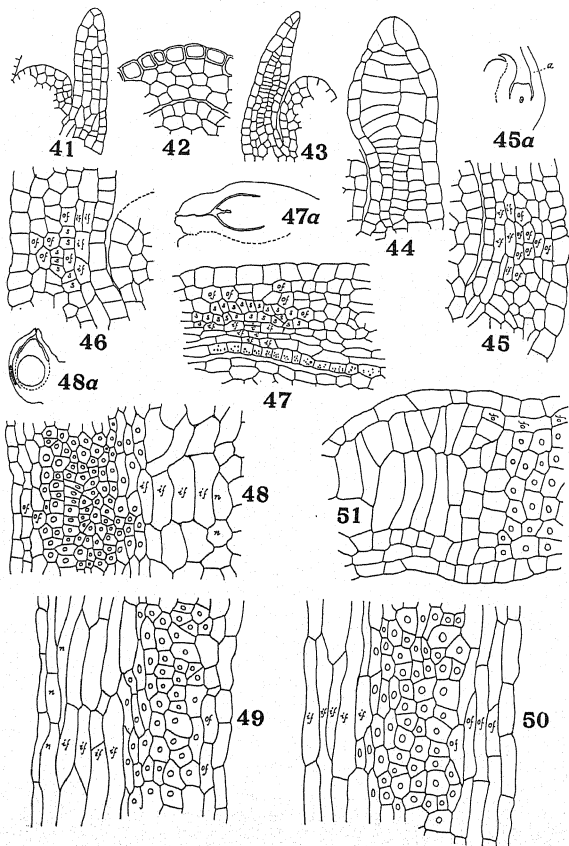
In *Pinus* the integument starts as a very simple structure, and, as in cycads and *Ginkgo*, its origin is hypodermal, beginning as two separate protrusions in a longitudinal section of the ovule, on both sides of the nucellus (fig. 41). As in cycads and *Ginkgo*, the nucellus appears first, but is overtaken very soon by the integument. The development of the integument from such a hypodermal layer until it exceeds the nucellus is similar to that in cycads. The development in cycads and *Ginkgo* is peculiarly a nastic movement, but in *Pinus* it is different. In young stages (fig. 41), before the megaspore mother cell is formed, the integument grows erect, and, as in cycads and *Ginkgo*, is at first homogeneous. In young stages it is composed of three rows of cells, two epidermal and the one at the center, the cells responsible for the formation of the different layers of the integument. The development of the integument from such a stage is accomplished by periclinal divisions, resulting in two homogeneous layers of cells. At the apex (fig. 41) the two mother cells still remain. The cells are more or less squarish and somewhat isodiametric. Following the periclinal divisions there are anticlinal divisions, the cells of the epidermis dividing most of the way anticlinally. The cross-section (fig. 42) is a replica of the longitudinal section. The cells are practically the same in size and are isodiametric. The walls adjacent to the outer epidermal cells are somewhat thickened.

The integument continues growing, and the tips show signs of bending inward (fig. 43). At this stage the outer row of cells has divided both anticlinally and periclinally, giving rise to long rows of prismatic cells; while the cells of the inner row of the integument remain dormant at this stage, so far as periclinal division is concerned. In the meantime the tip of the integument shows no

appreciable change; but a little later (fig. 44) periclinal division of the outer row of cells has taken place, the basal portion of the inner row remaining dormant and showing the beginnings of the differentiation of the outer and inner fleshy layers of the integument. The apical portion begins to show centripetal elongation. This behavior is preparatory to the bulging of the apex of the integument to close the micropyle, after pollen grains have reached the top of the nucellus. When the megaspore mother cell is formed, the outer and inner fleshy layers differentiate, and also show an increase in width. The left and right "arms" (seen in longitudinal section) of the integument occasionally show inequality of growth (fig. 45a). The tip of the integument nearest the ovuliferous scale curves somewhat, while the other one grows erect. Usually, however, the two "arms" grow parallel to each other (fig. 47a). A marked differentiation of the outer and inner fleshy layers soon appears (fig. 45), the outer being denser in cell contents, and with an abundance of resin, which is often so dense as to obscure the cell walls. The inner fleshy layer has lighter cells, however, with less cell contents, and the nuclei of the cells are very conspicuous. Periclinal divisions in both layers are evident. There are now four layers of isodiametric cells belonging to the outer fleshy layer. The inner fleshy layer cells are characterized further by being elongated longitudinally (fig. 45). Fig. 46 shows that belated condition in the development of the inner fleshy layer sometimes occurs. There is one distinct layer and the beginnings of the second layer in the inner fleshy layer, and the outer fleshy layer has undergone some differentiation. The beginning of the stony layer (fig. 46, *S*) is the result of anticlinal divisions of the innermost cells of the outer fleshy layer. As in *Ginkgo*, it shows that the stony layer originates from the innermost cells of the outer fleshy layer.

Like cycads and *Ginkgo*, the development of the embryo sac is also coincident with the development of the tissues of the integument. When the megaspore mother cell begins to divide, the stony layer, as well as the other layers on both sides, also develops. The tips of the integument have become nearly adnate (fig. 47a).

A further development of the stony layer (*s*) shows an increase in width, and although all cells are rather irregular in form, they are



FIGS. 41-51

generally isodiametric. The inner fleshy layer also keeps pace with the development of the other tissues of the integument. Three layers are now definitely formed, and there is the beginning of the fourth layer. While the embryo sac is in the free nuclear stage (figs. 48, 48a) the stony layer develops rapidly, the method of development being practically as in *Ginkgo*. The isodiametric cells are developed in the inner layer of the stony layer, but of course not as sharply isodiametric, because some cells are somewhat squarish. In cycads the isodiametric cells are developed on the exterior first, and have centrifugal development. The development of the inner fleshy layer affects, as in former cases, the innermost cells of the stony layer. These, by virtue of their position and proximity to the inner fleshy layer, elongate also (fig. 48). The inner fleshy layer cells are large and elongated longitudinally. The differentiation of stony cells continues to proceed outward and invades the outer fleshy

FIGS. 41-51.—Figs. 41-44, *Pinus Banksiana*: fig. 41, median longitudinal section of young ovule before pollination and formation of megaspore mother cell, showing details of arm of integument; micropyle open; integument still undifferentiated, $\times 166$; fig. 42, transverse section of ovule of same age as fig. 41, showing homogeneous nature of integument, $\times 195$; fig. 43, median longitudinal section of integument, showing early division of outer row of cells of undifferentiated integument, $\times 166$; fig. 44, median longitudinal section of integument, showing enlargement and elongation of apical cells before closing of micropyle, $\times 195$; figs. 45a-47, *P. excelsa*: fig. 45a, diagram of median longitudinal section of ovule, showing unequal arms of integument; megaspore mother cell already formed, $\times 27$; fig. 45, portion of arm *a* in fig. 45a, showing early differentiation of outer and inner fleshy layers, $\times 195$; fig. 46, portion of integument from median longitudinal section of ovule, showing belated condition of inner fleshy layer and early beginning of stony layer (*s*); stony layer originating from outer fleshy layer, $\times 195$; fig. 47a, diagram of median longitudinal section of ovule, showing two equal arms and stage when micropyle is closed, $\times 27$; fig. 47, portion of integument in fig. 47a, showing three rows of cells of inner fleshy layer, beginning of fourth, and developing stony layer, $\times 195$; figs. 48a-50, *P. Banksiana*: fig. 48a, diagram of median longitudinal section of ovule cut parallel to flat surface in free nuclear stage, $\times 6$; fig. 48, portion of integument in fig. 48a, showing 2 or 3 rows of cells of outer fleshy layer, very distinct thick stony layer, with transitional stages, and inner fleshy layer, $\times 195$; fig. 49, portion of integument in median longitudinal section of ovule cut perpendicular to flat surface, with 3 or 4 rows of inner fleshy layer cells, thick stony layer, and only one row of cells of outer fleshy layer, $\times 195$; fig. 50, portion of integument in median longitudinal section of ovule, when archegonium is formed, showing considerable outer fleshy layer, $\times 195$; fig. 51, *P. Laricio*: apex of integument in median longitudinal section, showing disappearance of inner fleshy layer in this region, profuse development of stony layer, and only one row of cells of outer fleshy layer, $\times 195$.

layer. Practically, at maturity, we have two or three layers belonging to the outer fleshy layer.

Sections made at different planes reveal different situations in the outer fleshy layer. If a longitudinal section is made parallel to the flat surface of the seed (fig. 50), a considerable outer fleshy layer is present. A section made perpendicular to the flat surface of the seed, however, shows a very little outer fleshy layer, often only one or two layers (fig. 49). In the mature seed of *Pinus Laricio* the outer fleshy layer is reduced to a thin dry membrane, but it does not lose its character of being made of several layers.

In cycads two systems of vascular bundles are present in the integument, one on the outside of the stony layer and the other on the inside. In *Ginkgo* the outer vascular system has disappeared, and this disappearance has resulted in a variation in the development of the stony layer. In *Pinus* both the inner and outer sets of vascular bundles have disappeared from the integument. This disappearance of the vascular system is interesting to note, and might throw some light on questions which will be discussed later. One conclusion which can be reached here is that lignification of stone cells is independent of any vascular bundle. The inner fleshy layer in *Pinus* has generally four layers of cells, but a fifth may occur. In a ripe seed it becomes reduced to a thin brown layer. While the middle and basal portions show such behavior, the apical region is also worth noting. The cells divide periclinally (fig. 51), but some remain elongated transversely. The inner fleshy layer disappears at the apex of the integument, while the outer fleshy layer shows its limits (fig. 51). The rest differentiates into isodiametric stone cells. In cycads and *Ginkgo* these apical cells of the stony layer become very much elongated longitudinally, as the inner fleshy layer.

While the characteristics of lignification are shown fairly well in cycads, the methods in *Pinus* are more conspicuous in the early beginnings than in either cycads or *Ginkgo*. No tannin or resin is present in the cells at this stage, but very early the outer fleshy layer becomes rich in resin, which seems to disappear at maturity. How the young unsclerified cells have used the resin is not known. In a stage of the ovule when the female gametophyte is already formed, the stony layer is distinguished from the other layers by the denser

contents of its cells. These cells are practically filled with cytoplasm more or less homogeneous, with very conspicuous and well organized nuclei. This is the situation at the middle region of the seed, but at a little higher level the cytoplasm becomes vacuolated (pl. fig. 14), and at the apical portion still more so. The dense portions around the vacuoles are made of different sizes of granular matter of unknown chemical composition. The writer is not now able to determine the morphological or chemical nature of these granules. Some of the nuclei of the cells begin to show a disorganization by the rupture of the nuclear membrane, allowing the nuclear material to ooze out. Cells have become pitted, while the nuclei are unchanged in a much higher level (near the apex of the integument) where lignification has started. The walls of the cells are thin, while those much higher are thicker (pl. figs. 14, 15). The thickening has begun to take place ahead of the pitting stage. While vacuolation is prominent here, the peripheral portion of the cells is more or less homogeneous, but rather dense. In these dense regions certain dark spots are observed, and between the dark spots pores are developed. The rest of the pitted mass seems to be connected with these dark spots, but the nature of the dense and thickened spots was not determined. From this stage pitting proceeds, and the process from this point is like that of cycads and *Ginkgo*. The mode of lignification is identical with that in cycads, starting from the micropylar end of the integument and proceeding downward. The chalazal development is rather belated, but also has a tendency to work upward. The innermost cells of the stony layer lignify first, and the process gradually proceeds outward, a replica of the cycad situation. While this is true, *Pinus* offers some puzzling variations from other types studied. In cycads and *Ginkgo* the stone cells in the ribs lignify also, are isodiametric, and have the same sculpturing as the ordinary outermost stone cells of the stony layer. In *Pinus*, however, cells in the ribs lignify and thicken, but the structure and sculpturing are entirely different (pl. fig. 16). The cells are isodiametric, but are of the smooth, simple sclerenchyma type, resembling bast fibers. In the young stages these rib cells are as thin as any outer fleshy layer cells, but generally without nuclei. Later they differ from the outer fleshy layer cells by acquiring double walls. These walls thicken

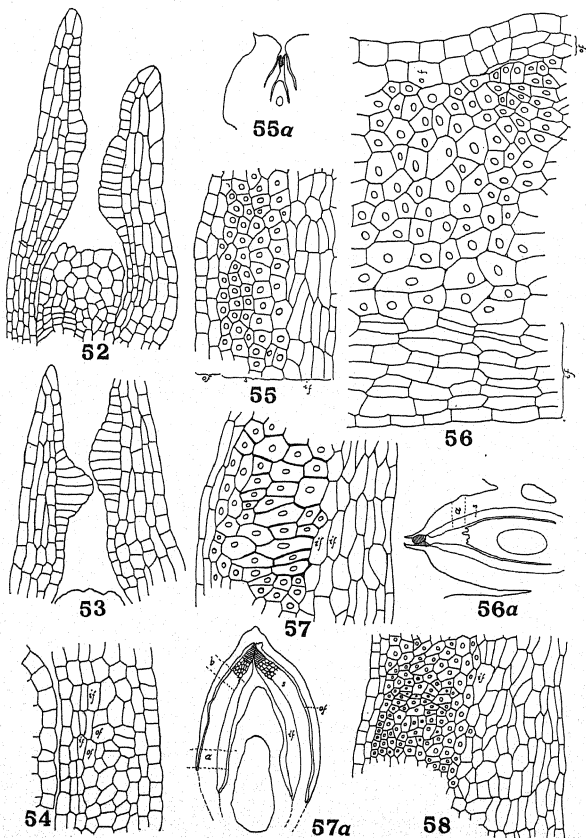
later like any sclerenchymatous cells of secondary wood, with no such cytoplasmic complexity as is seen in the case of stone cells. The ripe seeds split into two equal parts at these ribs, as is also true in two-ribbed seeds of *Ginkgo*. In a section parallel to the flat surface of the seed this is the structure that gives the appearance of a rather thick outer fleshy layer. Undoubtedly the rib cells belong to the outer fleshy layer. The nature of ribbing is very conspicuous here, and the ribs have no connection with the stony layer, while in cycads and *Ginkgo* these ribs are part of the stony layer.

In cycads and *Ginkgo* all cells of the innermost layer of the stony layer lignify and have practically the same structure, with little difference in the final sculpturing. In *Pinus*, however, all cells of the stony layer do not lignify, some in the outer layer remaining thin walled, and even the peripheral part of the walls of the already lignified stone cells does not lignify (pl. fig. 16). The cause of this behavior is not known, but the absence of bundles of any kind in the integument must be responsible for such variations. The origin and development of the stony layer in *Pinus* are much simpler than in the groups so far studied, but the nature of the stony layer in ripe seeds is complex. In cross-section (pl. fig. 16) the epidermis is thickened on the outside with a well formed cuticle. The two rows of outer fleshy layer cells are flattened, but they do not disappear as has been reported. Within the outer fleshy layer cells there are unlignified layers, usually two in the case of *Pinus Laricio*. This is a situation not met in any of the cycads or *Ginkgo*. These layers of thin walled cells belong to the stony layer. Within these thin walled cells we find the lignified cells of the stony layer. In a cross-section there are three types of cells. The outermost ones are U-shaped, with the outermost part of the peripheral walls unlignified; the middle layer is composed of the common isodiametric cells; and the innermost layer is composed of elongated longitudinal cells. The sculpturing of these lignified cells is the same as in *Ginkgo*, with fewer pits. In a longitudinal section of the seed, the two or three layers of the outer fleshy layer are more or less conspicuous, and the rest of the cells within are like those in the cross-section. The innermost lignified cells of the ribs are peculiar, with the innermost walls not thickened, and half-moon shaped, with tips connected (pl. fig. 16).

JUNIPERUS

The origin of the integument of *Juniperus* is analogous to that of *Pinus*, two protrusions, as seen in longitudinal section, coming out from the base of the nucellus (fig. 52). There is only one integument present, with an aril on each side (figs. 78, 79). As in *Pinus*, the integument is at first a homogeneous structure. The development of the integument, except at the apex, follows the same course as that described for *Pinus*. The apices of the two arms of the integument, as seen in longitudinal section, are far apart in the young ovule before pollination. In *Juniperus* the epidermal cells elongate centrifugally. In general the two arms, as seen in longitudinal section, dovetail together, but occasionally the arms meet in a straight line. The elongated epidermal cells do not divide periclinally, but retain the undivided type. When lignification starts in, these cells lignify first (fig. 57a).

The development of the integument is similar to that of *Pinus*. We have here also a variation common to *Pinus*, that often the two arms, as seen in the longitudinal section of the integument, are of unequal height and thickness. The twisting effect of one arm of the integument common in *Pinus* is not found here. The integument is homogeneous (fig. 52, left arm). In *Pinus* the outer and inner fleshy layers differentiate early at this stage, in the density and form of the cells. The right arm, however, shows at the bases of the integument the outer and inner fleshy layers differentiated. At the apex, where elongation of epidermal cells takes place, the cells are still homogeneous. At a little older stage, a week after pollination, when the primary nucleus of the pollen grain has divided, the epidermal cells elongate to close the micropyle. The epidermal cells below the swollen tip on both sides of the integument are filled with resin material which stains deep violet to red with the safranin, gentian violet, gold orange stain. The cells of the inner fleshy layer continue dividing periclinally, forming layers of parallel elongated longitudinal cells, a character which distinguishes the inner from the outer fleshy layer at this stage. The cells of outer fleshy layer also divide, but divisions are diagonal and anticlinal rather than periclinal. The outer fleshy layer shows the characteristic vertical and inclined walls (fig. 54), but the cells at the base of the integument are stretched. The two rows of cells (epidermal and subepi-



FIGS. 52-58

dermal) on the outside and also the inner epidermal cells are very densely impregnated with contents. Judging from this nature of the cells, the stony layer differentiates en masse, and the outer fleshy layer is limited to one layer only, adjacent to the epidermal cells. The outer fleshy layer of one row of cells is very marked in old ovules.

When the fleshy covering of the seeds grows and incloses the seeds, the stony layer also develops rapidly. The inner fleshy layer develops with parallel elongated longitudinal cells (figs. 55, 55a). The female gametophyte is in the free nuclear stage at this time. The innermost cells of the stony layer are isodiametric and the others are squarish. As the ovule develops (fig. 57a), the other layers of the stony layer stretch somewhat to elongated longitudinal cells (fig. 56); the other layers of cells continue dividing, and, although isodiametric cells are common, many are stretched transversely or with diagonal walls, especially near the apical region (fig. 57). The tip above the swollen region has three or four rows of cells in longitudinal arrangement, which remain as a needle-like structure at old age (fig. 56a). The outer fleshy layer is wider at the base of the integument, sometimes three or four layers of cells thick. There are also fewer cells with vertical or diagonal walls, the cells being mostly isodiametric and very much smaller than the isodiametric cells found above, or at the middle of the integument (fig. 58).

FIGS. 52-58.—*Juniperus virginiana*: fig. 52, median longitudinal section of ovule, showing two protrusions of integument, with undifferentiated tissue, $\times 166$; fig. 53, median longitudinal section of ovule, showing interior epidermal cells of integument elongating in process of closing micropyle, much older stage than fig. 52, $\times 166$; fig. 54, portion of integument in median longitudinal section, showing early differentiation of outer and inner fleshy layers, $\times 195$; fig. 55a, diagram of median longitudinal section of ovule, showing left arm of integument, $\times 6$; fig. 55, portion of left arm of integument in fig. 55a, showing one row of cells of outer fleshy layer, well developed stony layer, and inner fleshy layer, $\times 195$; fig. 56a, diagram of median longitudinal section of ovule, showing region *a*, $\times 6$; fig. 56, portion of integument *a*, fig. 56a, showing elongated longitudinal cells of inner fleshy layer, very thick stony layer, and one row of cells of outer fleshy layer, $\times 195$; fig. 57a, diagram of median longitudinal section of mature ovule in process of lignification, $\times 12$; fig. 57, details of *b*, fig. 57a, showing inner fleshy layer, isodiametric and elongated transverse cells of stony layer, and outer fleshy layer limited to one row of cells; also trend of lignification of cells, $\times 166$; fig. 58, details of *a*, fig. 57a, taken near base of ovule, showing type of stone cells, which are still parenchymatous, $\times 166$.

Lignification sets in at the time of the early formation of the archegonium initial. The elongated transverse epidermal cells of the tip lignify first, then the cells below them (fig. 57). Lignification starts from the outside and moves inward, from the apical region downward (fig. 57). The basal stone cells are belated (fig. 58). In old age one layer of the outer fleshy layer is recognizable (fig. 57) and is unlignified. The early stages of lignification are similar to those already described, with one exception. While pitting is quite universal in all genera studied, in *Juniperus* it is absent. The reticulated stage is the most prevalent and the most conspicuous. As in the other genera, the thickening of the walls is the first step; then reticulations come in. The absence of pitting in these stone cells seems to be related to the nature of the stone cells. In *Juniperus* they are rather simple (pl. fig. 17). While pores are present, they are fewer in number, simple in sculpture, and not forking. The thick walls show scattered and cut striations, not continuous throughout, and they have a few canaliculi. Both longitudinal and transverse sections of the old ovules with hard stony layer were examined and appear cycadean in nature, except the transverse inclined walls of the middle layer of the stony layer. The presence of fiber-like cells within the stony layer might be confused with the stony layer. Careful study of these cells shows that the fibrous cells, packed as they are, and with somewhat thickened walls, are the compressed, parallel elongated, longitudinal cells of the inner fleshy layer. They stain green and the stony layer red with safranin and light green stains.

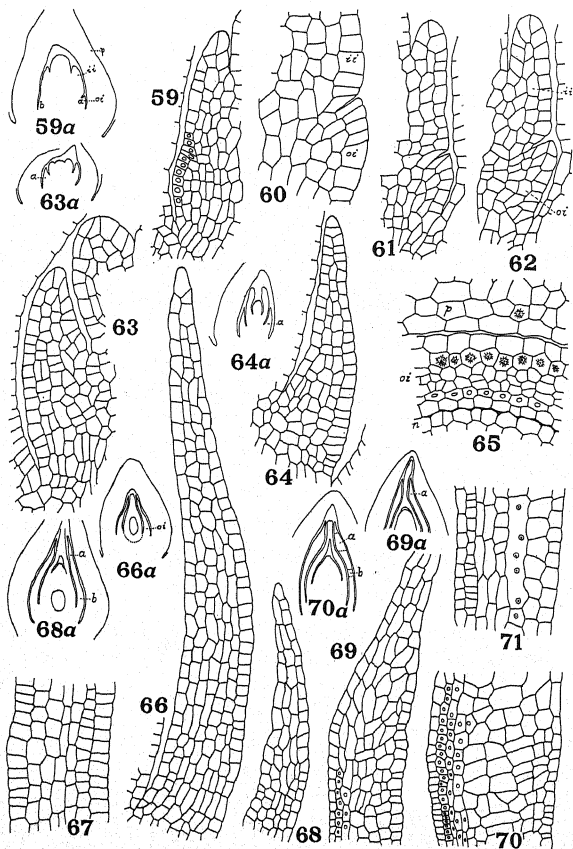
Gnetales

GNETUM

A study of the Gnetales is indispensable in this work. It not only affords excellent material for comparison, but also for critical study regarding the true nature of the female flower, and especially regarding the morphology of the envelopes. While the female flowers of Cycadales, Ginkgoales, and Coniferales have been the subject of extensive and intensive studies, the Gnetales, although comprising but three genera, have assumed great importance because of a possible relation with the angiosperms. Naturally the Gnetales, especially *Gnetum*, have received very critical consideration.

Despite the voluminous literature now available, the stony layer, its origin, development, and nature have not received much attention. The stony layer has been valuable in the interpretation of other tissues of the ovules in the preceding groups, and so the Gnetales were included in these studies. The female flower of *Gnetum* has two integuments, an outer and an inner one. The outer fleshy covering may be called the "perianth."

The integuments of the ovule show structures and variations which will require comparative and more critical studies. In various species of *Gnetum* the nature of the integuments is different, some having the outer integument adnate to the perianth, others separated; in some the outer integument is adnate to the inner integument half the length of the ovule, and others adnate at the base only. It is to be regretted that many species previously studied are not within my reach at this time. For young stages until the lignification stage, ovules of *G. Gnemon* were studied. The origin of the integuments has been studied by many botanists in conjunction with morphological studies of the female gametophyte. There are three views regarding the origin of the integuments. The diversity of opinion is probably due to the many species studied, or to variations in the same species. The writer has found that even in the same species the origin of the integuments varies. In *G. Gnemon* the perianth is developed first in the life history. The growth of the integuments is basipetal (fig. 59a). Near the base of the inner integument some cells become specialized, first by enlarging and then by dividing periclinally (fig. 59). The development of the two arms (longitudinal section) of the outer integument is not simultaneous; one arm grows faster than the other (figs. 59; 60), and the unequal length of the arms is evident even in the old ovules. After the periclinal division the cells seem to twist, and the first visible sign of separation of the outer from the inner integument is the presence of a crack, which is formed by the tendency of the outer integument to grow outward. It is evident that the outer integument arises from the basal portion of the inner integument (figs. 60-62). After the first sign of separation the cells divide radially, in a more or less periclinal direction. While the basal cells of the inner integument become dormant, the cells of the juvenile outer integument grow



FIGS. 59-71

very rapidly (fig. 62). Morphologically the outer integument belongs to the inner integument, judging from its origin; but later in its ontogeny the two integuments separate.

The development of the inner integument will not be considered in this discussion, except that it grows out as the micropylar tube in old age. The development of the outer integument and its nature in the mature stage will throw more light on the interpretation of the number of the integuments. While the growth succession of the integuments in the case of *Gnetum Gnemon* is basipetal, somewhere in the life history, after the megaspore mother cell is formed, a belated condition of the development of the inner integument may be observed (fig. 63). This variation, although not very uncommon, has probably led STRASBURGER, LOTSY, and THOMPSON to advance

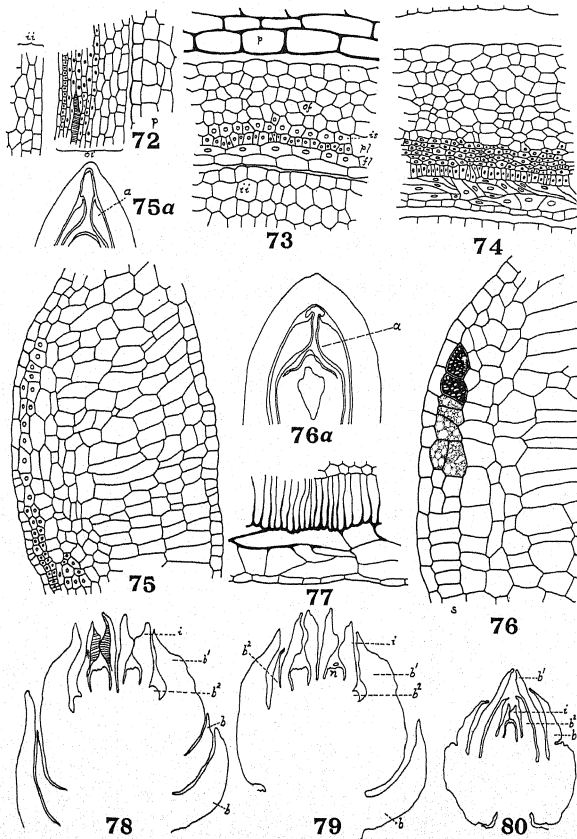
FIGS. 59a-71.—*Gnetum Gnemon*: fig. 59a, diagram of median longitudinal section of young ovule in early development of outer integument (*oi*) from basal region of inner integument (*ii*); perianth (*p*) covers ovule, $\times 13$; fig. 59, details of *b*, fig. 59a, showing origin of outer integument, $\times 502$; fig. 60, details of *a*, fig. 59a, showing early development of outer integument; note mode of splitting, $\times 502$; fig. 61, portion of median longitudinal section of ovule, showing inner and outer integuments; much older stage than fig. 60, $\times 300$; fig. 62, portion of median longitudinal section of ovule in megaspore mother cell stage, showing inner and outer integuments; much older stage than fig. 61, $\times 300$; fig. 63a, diagram of median longitudinal section of ovule, showing much more developed outer integument, $\times 27$; fig. 63, outer integument in *a*, fig. 63a, showing type of cells and their development, $\times 300$; fig. 64a, diagram of median longitudinal section of ovule, showing ordinary type of development of integuments, $\times 27$; fig. 64, outer integument in fig. 64a (*a*), showing early differentiation of outer and inner regions of integument, $\times 300$; fig. 65, portion of transverse section of ovule in very early free nuclear stage, showing cells of outer fleshy layer with calcium oxalate crystals, and inner region of outer integument, $\times 195$; fig. 66a, diagram of median longitudinal section of ovule in free nuclear stage, showing outer integument, $\times 27$; fig. 66, outer integument (*oi* in fig. 66a), showing elongated longitudinal cells of inner region and early beginning of isodiametric cells, $\times 195$; fig. 67, outer integument near base of ovule (fig. 68a), showing differentiation of outer fleshy and stony layers, $\times 300$; fig. 68a, diagram of median longitudinal section of ovule with inner and outer integuments, $\times 13$; fig. 68, apex of outer integument in fig. 68a (*a*), $\times 166$; fig. 69a, diagram of upper half of median longitudinal section of ovule, showing region *a*, where details in fig. 69 were taken, $\times 6$; fig. 69, apex of outer integument in fig. 69a (*a*), showing beginning of differentiation of two layers of stony layer, $\times 166$; fig. 70a, diagram of upper half of median longitudinal section of ovule, showing regions *a* and *b* of outer integument, $\times 6$; fig. 70, outer integument near apex in fig. 70a (*a*), showing further development of innermost layers of stony layer, $\times 166$; fig. 71, details from *b*, fig. 70a, showing similar differentiation as in fig. 70, and also some cells of outer fleshy layer without cross-walls, $\times 195$.

the view that the integuments develop in acropetal or centripetal succession. The most usual way, however, is basipetal (fig. 64). The early development of the outer integument is complex. The cells are rather isodiametric, but signs of parallelism of cells may be distinguished very early (fig. 62). Although the development of the outer integument has been investigated by several botanists, a detailed account of the development of the outer integument is lacking. The cells of the outer integument are isodiametric at first, but soon show the parallel elongated type common in the Coniferales (fig. 64). There is no inner fleshy layer of the outer integument. The outer integument differentiates as an outer fleshy layer and an inner region which later will become the stony layer (fig. 64). As in the case of all of the species studied, the apical portion shows slow progress in the differentiation to outer fleshy layer and the inner region. In cross-section (fig. 65) the homogeneity of the tissue is quite evident. Cells are generally isodiametric, while the innermost cells become later the palisade and fibrous layer, differentiated here as a single layer of cells more or less elongated and with rather scanty contents. The outermost subepidermal layer is characterized by the presence in each cell of calcium oxalate crystals. As the outer integument grows in both length and diameter (much more rapidly longitudinally), all cells become practically parallel, except a few in the outer region. The first step in the differentiation of the fibrous layer is the anticlinal division of the cells (figs. 66, 66a). A more detailed study of this differentiation, when the female gametophyte is in the free nuclear stage, is seen in fig. 67, where the fibrous layer has divided anticlinally and the palisade region is beginning to divide in a similar direction. The trend of such activity is acropetal. While the cells of the base react in that way, those at the apical region begin to enlarge slightly, with anticlinal and radial divisions (figs. 68, 68a). The apical growth becomes active when the ovule assumes a larger size (fig. 69a), and differentiation of the outer fleshy region and the stony layer takes place. The fibrous layer of the stony layer differentiates from the rest of the elongated longitudinal cells by anticlinal and radial division (fig. 69). The cells adjacent to this layer toward the outside are still elongated longitudinally, but in the mature stage will become the palisade layer. Further develop-

ment of the apex of the outer integument leads to further differentiation of its various cells (fig. 70a). At the tip, two-thirds of the outermost cells enlarge enormously and also elongate. The innermost cells continue dividing anticlinally, and the next layer of thin elongated longitudinal cells shows beginnings of anticlinal division (fig. 70). Somewhere near the base and the middle region the cells of the elongated longitudinal type divide anticlinally. The beginning of the anticlinal division of the elongated longitudinal cells toward the formation of the isodiametric type cells of the stony layer is well marked (fig. 71). The innermost layer of the stony layer is the first to differentiate, first into the isodiametric type and then to the elongated longitudinal type at maturity. The formation of the palisade type cells of the stony layer is belated. Incidentally, while all this is taking place, dissolution of the cross-walls of some cells of the outer fleshy layer of the outer integument is quite common (fig. 71). The vascular bundle of the outer integument traverses the stony layer outside of the palisade region, in the isodiametric type region (fig. 72). No vascular bundles are present in the young ovules, but they appear soon in the mature ovules before the formation of the palisade layer.

The next step in the development of the outer integument is the formation of the isodiametric cells of the outer fleshy layer and those of the outer layer of the stony layer (fig. 73). The palisade layer is formed by anticlinal division of the parallel elongated longitudinal cells within the subepidermal layer, which is the last to mature. Very often the cells divide periclinally and form two to four rows of cells (fig. 74), but occasionally there is only one row, with cells failing to divide periclinally. In *Gnetum Gnemon* the subepidermal cells do not elongate longitudinally (figs. 75, 76, 76a). The cells of the outer fleshy layer of the outer integument elongate transversely. At the apex the cells are somewhat irregular in shape, although a more or less isodiametric form is quite common on leaving the apical region and proceeding downward. The stony layer below level *a* (fig. 75a) is differentiated into three distinct layers, the isodiametric layer outermost, the palisade layer in the middle, and the fibrous layer innermost.

Lignification does not start at the very tip of the outer integu-



FIGS. 72-80

ment, but begins somewhere near the base of the tip and proceeds downward (figs. 76, 76a). The chalazal lignification is very much belated. In the mature ovule, however, lignification extends to the very tip. Lignification starts first in the outer walls of the palisade layer (fig. 77) and not in the fibrous zone; then the side walls of the palisade layer lignify, and the process extends outward to the isodiametric cells. Lignification of the innermost fibrous layer of the stony layer is simultaneous with lignification of the outer walls of the palisade cells. The fibrous layer is generally five-angled at the tip. Lignification of the inner epidermal cells is rather belated and comes last in the history (pl. fig. 18). The method of lignification is identical with that of *Pinus*.

There are three distinct layers of cells belonging to the stony layer. In the case of *Gnetum Gnemon* there are three or four layers of isodiametric cells. Sometimes there are but two, but never less. There are five or six pores in each cell, and these pores connect with the adjacent cells. The pores are simple, and not forking, and rather enlarged and club-shaped at the ends adjacent to the middle

FIGS. 72-80.—Figs. 72-77, *Gnetum Gnemon*: fig. 72, portion of outer integument in median longitudinal section, taken at middle, showing vascular bundle traversing stony layer, differentiation of different layers of stony layer, and beginning of isodiametric cells; from ovule 6.25×3.5 mm., $\times 195$; fig. 73, portion of outer integument in transverse section, showing perianth (*p*) on outside and various layers; stony layer shows early differentiation of isodiametric cells, palisade layer, and fibrous layer before dividing periclinally, $\times 195$; fig. 74, portion of outer integument, showing more developed stony layer with several rows of isodiametric cells; palisade and the fibrous layers well differentiated, $\times 166$; fig. 75a, diagram of upper half of median longitudinal section of ovule, showing region *a* of outer integument, $\times 6$; fig. 75, apical region of outer integument of ovule in fig. 57a, showing type of cells, $\times 166$; fig. 76a, diagram of upper half of median longitudinal section of mature ovule, showing region where details in fig. 76 were taken, $\times 6$; fig. 76, details of *a*, fig. 76a, showing start and trend of lignification; two cells above showing pitting stage and those below vacuolated stage on way to pitting, $\times 166$; fig. 77, transverse section of portion of stony layer, showing few isodiametric unligified cells, palisade cells beginning to lignify, and fibrous layer, $\times 166$; fig. 78, *Juniperus virginiana*: diagram of median longitudinal section of young ovule, showing basal sterile bracts (*b*), fertile bract (*b'*) which later covers ovule, undeveloped bract (*b''*) often called aril, and single integument (*i*), $\times 12$; fig. 79, *J. virginiana*: diagram of median longitudinal section of young ovule about same age as in fig. 78, with same parts, except that portion of aril (*b''*) has developed somewhat, $\times 12$; fig. 80, *Torreya taxifolia*: diagram of median longitudinal section of ovule with envelopes; so-called outer integument (*b'*) nothing but bract comparable with aril of *Juniperus*, $\times 6$.

lamellae. The inner pores of the isodiametric cells connect with the single pores of the palisade stone cells. The cells of the second type belonging to the second layer of the stony layer are palisaded. As in isodiametric cells, there are radiations, but they are parallel to the sides. The third innermost layer of elongated longitudinal cells is often two-layered, but three or four layers are not very uncommon in the "fibrous layer." Radiations in the cells are fewer and simple, and in gross examination they appear solid. In the palisade and fibrous layer no transverse pores are found. The innermost epidermal cells of the outer integument also lignify, but are rather belated in the history. The lumina of the cells are small.

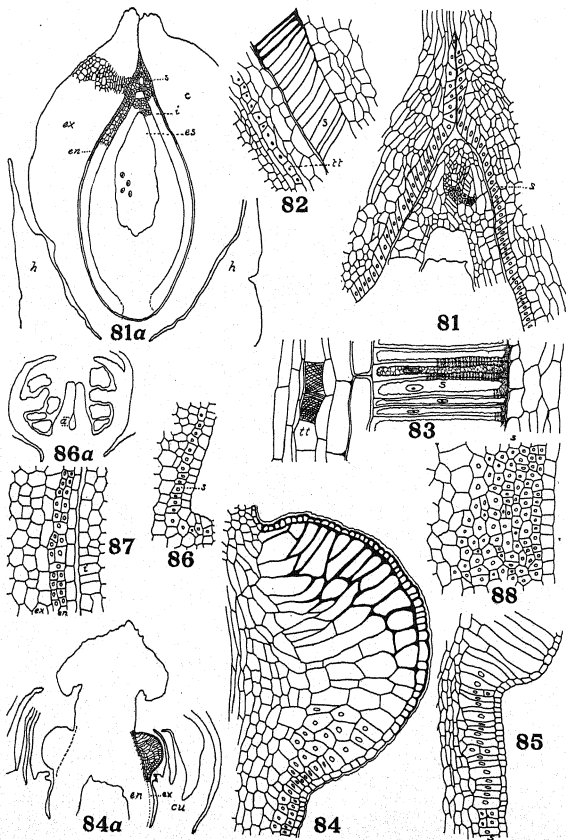
Angiosperms

The study of the origin and development of the stony layer in angiosperm seeds is very interesting, taking into account the variations that this group affords. Realizing the value of the study of the stony layer in seeds of angiosperms, the writer will mention the subject here, not as a supplement but rather as an introduction. A more complete report will follow in the near future. The present statement includes preliminary studies on the stony layer in *Sassafras sassafras*, *Quercus velutina*, and *Prunus pennsylvanica* (var. Early Richmond). The material of *Q. velutina* was collected on the University of Chicago campus, and the other two species from Smith, Indiana. The collections cover a period of more than a year (two summers, two autumns, a winter, and one spring) at weekly intervals. The sequence of genera is according to the nature of the stony layer and not phylogenetic.

The origin of the stony layer depends upon the morphological nature of the flower. It may arise from the innermost or outermost region in a flower of carpellary nature; or it may arise from the interior of a torus, etc. The trend of development is from inside outward in case of inward origin; or it may develop from outside to inside. The increase in thickness of the stony layer may be due to elongation of the cells without division, or may be due to either periclinal or both periclinal and anticlinal divisions of the cells. In *Sassafras sassafras* the origin of the stony layer is epidermal. The epidermal cells do not divide, but differentiate later into the stony

layer. This is the simplest case studied. In the very young stages the stony layer is not differentiated from the rest of the cells, except that it is epidermal; but soon, when the megaspore mother cell is formed, the innermost epidermal cells of the carpel differentiate by increasing in size and by elongation. The megaspore mother cell is also differentiated from the other adjacent cells by the presence of definite and well marked nuclei, and dense contents. The development of the megaspore mother cell is accompanied by the development of the stony layer also. Instead of dividing periclinally, as in other stony layers, the cells simply elongate. The elongation starts from the apical region and proceeds downward (figs. 81a, 81). This is very evident even before fertilization. In older stages the cells are palisaded and unicellular. Lignification starts after fertilization, and is quite gymnospermic in nature, pitting being a conspicuous stage. In the very old stone cells, pits remain and even thick walls remain pitted (fig. 83). The nuclei in the stone cells persist. Transfusion cells are present in the integument and extend all round the integument (figs. 82, 83). The transfusion cells also differentiate very early in the life history. Reticulations of the transfusion cells appear when the embryo is already formed (fig. 83). The drupaceous fruit has a fleshy exocarp and a stony endocarp.

The stony layer of *Quercus velutina* is as simple as that of *Sassafras sassafras*, although slightly more complex than the latter, because, instead of being unicellular, the bicellular state is sometimes preserved, even in old stone cells. The stony layer is differentiated very early also. Instead of arising from the inner region of the carpel, the outer subepidermal layer differentiates, first by elongating and later dividing periclinally. The cells of the style remain small and isodiametric, but at the base at the "wings" they divide periclinally, enlarge, and elongate. The winged condition of the carpel at the apical region recalls the situation of the apical region of the arms of the integument in *Pinus* (figs. 84, 84a). The elongation and enlargement of the cells have a downward trend. The two rows of subepidermal cells are rather squarish and isodiametric at the start, but they soon elongate transversely when the flower develops (fig. 85). Those within begin to enlarge and often elongate longitudinally. At the base of the ovary several layers of cells of the sub-



FIGS. 81-88

epidermal layer are homogeneous, small, and often isodiametric. In a much older stage (fig. 85) the cells of the wing elongate enormously while those below follow. The stony layer is generally two-celled in longitudinal section, resulting from periclinal divisions in the young stage; but in the mature stages the cross-walls disappear, and the old stone cells seem to be unicellular as in *Sassafras sassafras*. The mature acorn is composed of a heavily cutinized epidermis, stony exocarp, and a fleshy thin endocarp, which later become dry and papery. The stone cells at the chalazal region are generally isodiametric. They have smooth thick walls, with no pores or pits. Lignification starts from the apical region at the wings and proceeds inward (fig. 84). Some cells at the base of the bracts which form the cupule lignify also, and show an interesting structure. There are enormous branching pores, which resemble some of those in stone cells of cycad seeds (fig. 84a).

Prunus pennsylvanica shows a stony layer with two distinct layers. Instead of having a single palisade stony layer, this layer is quite differentiated. When the flowers are still in the bud condition, in the early formation of the ovules, the stony layer is not differentiated from the rest of the inner carpellary tissue (figs. 86a, 86). It arises first as a differentiated layer in the innermost region of the carpel (fig. 86), the cells being isodiametric and subepidermal in

FIGS. 81a-88.—Figs. 81a-83, *Sassafras sassafras*: fig. 81a, diagram of median longitudinal section of young fruit in proembryo stage, showing carpel (*c*) with epidermis which differentiated as stony layer (*s*), embryo sac (*es*), integument (*i*), and husk (*h*), $\times 27$; fig. 81, apical region of fruit, showing stony layer and transfusion tissue in integument in very young stage, $\times 166$; fig. 82, apical region of fruit, showing stony layer and much more developed transfusion tissue (*u*), $\times 166$; fig. 83, stony layer in median longitudinal section, showing nature of stony layer and reticulations in transfusion tissue (*u*), $\times 300$; fig. 84a, *Quercus velutina*: diagram of a median longitudinal section of very young acorn, showing wing and bracts which form cupule (*cu*), $\times 12$; fig. 84, *Q. velutina*: wing in fig. 84a, showing origin of lignification of cells and stony layer; cells with nuclei, $\times 166$; fig. 85, *Q. velutina*: endocarp (*en*) and exocarp (*ex*); cells with nuclei form stony layer and also exocarp, $\times 195$; figs. 86a-88, *Prunus pennsylvanica*: fig. 86a, diagram of median longitudinal section of very young flower, showing carpel before ovule is formed, $\times 47$; fig. 86, details from region *a*, fig. 86a, showing undifferentiated carpel and origin of stony layer (*s*), $\times 195$; fig. 87, portion of carpel from very much older flower, showing two rows of cells belonging to endocarp (*en*), $\times 300$; fig. 88, portion of carpel from flower much older than fig. 87, showing several layers of cells belonging to endocarp, which later become stony layer, $\times 195$.

position. These cells soon divide periclinally (fig. 87), and the successive divisions make the stony layer a differentiated tissue, and incidentally differentiate the exocarp from the endocarp (fig. 88). The stony layer at first is undifferentiated, composed wholly of isodiametric cells, but as the fruit develops the stony layer also differentiates. At the apical region the cells are isodiametric, and at the middle level the cells are also isodiametric, but some are slightly elongating longitudinally. At the base the cells are generally isodiametric. Near the periphery of the stony layer they are smaller, and larger toward the outer region. In transverse section the inner layer of the stony layer is made up of thin, elongated longitudinal cells, and the outer layer of rather large isodiametric cells. The method of lignification is like that in most gymnosperms. Pitting is very conspicuous, and, as in *Sassafras*, pits are present even in the old stone cells. The sculpturing of the walls resembles very much that of the cells in pl. fig. 10. Lignification starts from the outside and has an inward trend. The outer cells have generally thicker walls than those of the inside. The dissolution of the cellulose in the exocarp in ripe fruits is very evident, and is changed to pectic substances. Whether these pectic substances play some rôle in the lignification and thickening of the walls of the stone cells, we are not yet ready to state.

Discussion

The stony layer of the seeds of gymnosperms has been a very important factor in the interpretation of tissues. The study of the stony layer in these seeds showed its origin, development, and nature. From this it was possible to solve some of the problems in regard to the true nature of the envelopes of the ovule.

The cupule of *Lagenostoma* seeds was studied and was compared by STOKES (58, 59) to the testa of the cycad ovule. She concludes that the cupule of the *Lagenostoma* seed shows all the features of vascular anatomy of the outer fleshy layer of the cycad ovule. From this she advanced the view that the outer fleshy layer of the cycad ovule is an integument, so that altogether there are two integuments. She also noted the point of fusion of the two integuments. While the vascular situation may throw light, it cannot be

used as a criterion. Her failure to study the stony layer from its origin led to an erroneous interpretation. The origin and development of the integument show that it is a single structure beginning as hypodermal cells, constituting a homogeneous tissue. The cycad ovule has only one integument. LAND (28), in his studies of *Ephedra*, made a generalized statement that "the tendency in both gymnosperms and angiosperms seems to be toward union of the two integuments into one." There is no doubt that *Ephedra* and *Gnetum* have two integuments. While this is true, it cannot be inferred that other gymnosperm seeds also have two integuments, but that, instead of being separated, they are united. Also the presence of two integuments cannot be assumed as a primitive character. LAND remarked that in Taxineae there are two integuments, and regarded the situation as primitive. My studies of the Coniferales (*Pinus*, *Juniperus*, and *Torreya*) seem to disprove this view. In Abietineae (*Pinus*) there is but one integument differentiated into three layers, an outer fleshy, a stony, and an inner fleshy layer. In most cases and in old age the outer and inner fleshy layers are very inconspicuous, the outer fleshy layer being reduced in thickness to one, two, or three rows of cells, and the inner fleshy layer often converted into a dry papery membrane. The stony layer is the principal one. Studies of *Juniperus* show that the ovule has one integument. Very often at the base of the integument two protuberances are present, which have been designated by several botanists as an aril. Occasionally, however, one of them develops, but never exceeds the integument. The occasional development of the aril is interesting and shows that it is nothing more than an undeveloped bract. The fleshy covering of the seeds is the bract. This behavior brings us to the situation in Taxineae (*Torreya*). It is the current opinion that *Torreya* has two integuments. The so-called outer integument is belated in growth and has been designated variously as outer integument, aril, and epimatium. According to COULTER and LAND (17), the outer integument develops the thick fleshy covering of the seeds, while the inner integument differentiates into two layers, the outer of which is stony while the inner consists of thin walled cells. This is practically the replica of *Juniperus* features. While the vascular bundle situation would give support to the views of these investi-

gators, studies of the stony layer in these seeds seem to show that the so-called inner integument of *Torreya* is homologous with the single integument of *Juniperus*. The development of the integument tissue closing the micropyle in *Juniperus* is duplicated by the so-called inner integument of *Torreya*. From this feature and the nature of the stony layer it is very evident that *Torreya* has but one integument, the so-called aril in *Juniperus*, which has developed a function different from that of an aril. This is not the case in Gnetales. In *Gnetum* there is no doubt that the outer integument originated from the inner. While the integument is single at the start, it differentiates later in the life history into two separate integuments.

The cupule of *Lagenostoma* is really the homologue of a swollen portion which appears at the base of some cycad ovules. The stony layer is epidermal in origin, with a hypoderm within comparable with the inner fleshy layer of cycad or *Ginkgo* ovules. The peglike outgrowths of the epidermal walls are nothing but specialized walls, or probably the remains of the epidermal walls, and in that case the stony layer is hypodermal in origin. The fact that the features of the vascular bundle of the cupule of *Lagenostoma* are identical with those of the outer fleshy layer of the cycad ovule should not be too strongly urged in support of the view that the two are homologous. The situation in *Ginkgo* follows the same trend. The outer fleshy layer of the ovule is devoid of any vascular bundle, which is formed only in the inner fleshy layer of the integument. This variation affects the origin of the stony layer. Instead of being made of cells of the outer and inner fleshy layers, it is composed wholly of cells of the interior of the outer fleshy layer. The presence of two bundles in the inner fleshy layer and the outer fleshy layer of cycad ovules is the result of the forking of the main bundle entering at the base. In *Ginkgo* the bundle failed to fork. The aril or collar of *Ginkgo* is an extra envelope which failed to develop, and is not an integument. The cupule of *Lagenostoma* is the homologue of the collar of *Ginkgo*, the bract of *Pinus*, the aril of *Juniperus*, the so-called outer integument of *Torreya*, and the "perianth" of *Gnetum*. In cycads the swelling at the base of the ovule, especially in *Cycas*, may be regarded as homologous with the undeveloped aril of *Ginkgo*. From the fore-

going facts, one can establish the continuity of tissues, except in *Lagenostoma*, which has a well developed cupule already. In *Pinus* there is but one integument, and it is unprotected. The collar of *Ginkgo* is the vestige of the cupule of *Lagenostoma*, and is homologous with the bract in *Pinus*. The integument has no bundles. In *Juniperus* the development of the envelopes is basipetal. The single integument is developed first. The so-called aril is usually inactive and undeveloped. The bract develops later and becomes the fleshy covering of the seed. The integument, as in *Pinus*, has no bundles. The aril of *Juniperus* is homologous with the collar of *Ginkgo*. In *Torreya* there is also but one integument with no bundles. The aril which envelops the seed later is homologous with the aril of *Juniperus*, and the extra bract is homologous with the fleshy covering of *Juniperus*. In *Gnetum* there are two integuments, the outer originating from the inner, and both are provided with bundles. The growth of the integuments showing a basipetal succession is interesting to note. The collar of *Ginkgo*, the aril of *Juniperus*, and the so-called outer integument of *Torreya* are homologous with the perianth of *Gnetum*.

The possible relation to the situation in angiosperms is suggestive. If an homology is possible, the so-called outer integument of *Torreya* and the perianth of *Gnetum* are homologous with the carpellary wall of the fructifications of *Sassafras*, *Quercus*, *Hicoria*, and *Prunus*. The cupule in *Hicoria*, *Quercus*, and *Corylus* is an extra envelope. In *Hicoria* it is composed of a fused solid bract, while in *Quercus* and *Corylus* the cupule is composed of bracts.

The cause of lignification is an unsolved problem, and the process of lignification is even more complex. Is the lignification of the cells at a certain region of the integument due to difference in water content in the cells, to diffusion of colloids, or to the transformation in the stone cells of one chemical substance into another? While no extensive experiments have been made to determine these various factors, an attempt will be made here to interpret the method of lignification, with the help of anatomical evidence. The decrease of water content in the stone cells in old age is probably related to the lignification of cells. In very young stages the cytoplasmic materials of the stone cells are homogeneously scattered. The develop-

ment of the stone cells is accompanied by an increase in granular matter in the cytoplasm of the cells, not only in size but also in number. Ordinarily the dense part of the cytoplasm is observed near the walls, where the material is piled. Vacuolation, the second stage, is probably due to the diminution of water content and to the aggregation of the granules. Vacuolation is a very effective phase during lignification. After the vacuoles are fully formed, the granules seem to disappear as the tissue again appears homogeneous. Pitting is quite common in some cases, but in others it is altogether absent, and in still others it remains as a feature of the adult stone cells.

Various views as to the nature of the lignin have been proposed. The old idea is that lignin is cellulose encrusted with some substance. Miss ECKERSON remarked, in one of her lectures in plant microchemistry, that on hydrolysis of lignin a number of aromatic fatty acids are obtained. She thought that lignin is an ester of cellulose and aromatic fatty acid. In the ascospore of *Peziza* the young spore wall is formed from the plasma membrane. The piling of dense protoplasm in early lignification may be called "Hautschicht." When the precipitate is a pectic substance, that substance is transformed to another layer of cellulose. These different layers of cellulose later become lignified, and the striations seen in the thick walls are nothing but these layers of cellulose piled on the original cellulose walls. These walls are properly formed by precipitation and chemical reaction. The change of cellulose to lignin, according to some chemists, is due to an intramolecular change of cellulose. This change is very evident in the cherry, where the cellulose walls are transformed to pectic substances during lignification and while ripening. Whether the pectic substances are again synthesized into cellulose and into stone cells, or whether the change is from a hexose to a pentose, is difficult to ascertain now. According to THATCHER (62) and various others, the cellulose is converted to ligno-cellulose. How ligno-cellulose is formed is at present difficult to understand. According to all chemical studies, the stony layer is made up wholly of lignin, with no trace of chitin, cutin, or suberin.

In young ovules the integument is green, and is well provided with chlorophyll, and in older stages starch abounds; but when lignification begins, starch seems to disappear. In *Pinus* especially,

the stony layer is the dense portion before lignification. Whether the starch is converted to a hexose sugar, and this later converted to a pentose sugar is hard to tell at present.

In young stages the stone cells are often impregnated with either tannin or resin. In *Pinus* the resin in the stone cells seems to disappear when the cells lignify. What significance this disappearance may have would be a difficult thing to solve. In cycads also some stone cells are impregnated with tannin. The fact that tannin persists in the cells, even when they are hard and lignified, shows that it plays no part in the lignification of cells. Lignification is also independent of bundles, since cells lignify in regions where bundles are absent.

The important question is why the development of the stony layer is localized in the ovules. It is generally conceived that cells lignify to protect the internal organs of the ovule, but why they lignify there is not known. No doubt the position of the stony layer is phylogenetic and is constant in each group. In all species studied lignification starts from the apical end and proceeds downward. The only exception to this rule is *Ginkgo*, in which lignification starts at the chalazal end, and this variation is accounted for by the presence of transfusion tissue at the base of the ovule. The presence of this tissue around the integument of the *Sassafras* ovule is probably responsible for the epidermal origin of the stony layer. So far as I am aware, it is the only genus in angiosperms with such a behavior. In cycads and *Ginkgo* the lignification starts from within. In *Pinus* not all cells and walls belonging to the stony layer lignify; the outer part of the stony layer remains parenchymatous, while some of the cells of the outer lignified layer do not lignify, but become U-shaped. The development of lignification is cycadean. *Juniperus* is like *Pinus* in all respects, with the exception that cells on the outside lignify first and then lignification proceeds inward. In *Gnetum* the basipetal mode of lignification is natural, but the middle layer (palisade) lignifies first, and the outer and the inner layers lignify practically simultaneously. This variation is probably due to the chemical condition of the integument, and also to the morphological nature of the ovule. In ovules with no extra envelope the lignification from within is universal; but in ovules with a fleshy covering, like those of *Juniperus* and *Gnetum*, such variation in lignification is

evident. In the angiosperm seeds studied the basipetal mode of lignification is universal, and that lignification starts from the outside and proceeds inward is very evident.

Summary

1. The stony layer in seeds of gymnosperms is integumental in origin as far as the integument extends, and in the angiosperms studied it is carpellary. In cycads both the outer and inner fleshy layers contribute to the formation of the stony layer. In *Ginkgo*, *Pinus*, *Juniperus*, and *Torreya* the stony layer originates from the outer fleshy layer. In *Gnetum* it originates from the inner region of the outer integument. In *Sassafras* the stony layer originates from the interior epidermal cells of the carpel. In *Quercus velutina* it is the exterior subepidermal layer that differentiates into the stony layer. In *Prunus* the interior subepidermal layer of the carpel divides and forms the stony endocarp.

2. There are simple and complex stony layers. In cycads the stony layer is made up of isodiametric cells and elongated longitudinal cells. In *Ginkgo* the situation is the same. In the Coniferales there is the start of the complex stony layer. There are isodiametric cells, elongated transverse cells, elongated longitudinal cells, and cells partly lignified, and still others with parenchymatous cells. In *Gnetum Gnemon* there are three types of cells; besides the two found in cycads, there are also palisade cells. Stone cells are pitted and non-pitted; also there are stone cells with pores, branching pores, and without pores; and also cells with and without striations. In the angiosperms there are also simple and complex stony layers.

3. The direction of the progress of lignification varies according to the structure of the ovule.

4. The chemistry of lignin is very complex, and the nature of lignification might best be solved by the aid of morphological data.

5. The stony layer seemed to be connected with the phylogeny of the groups in gymnosperms. In the angiosperms studied further investigation will determine the relationship.

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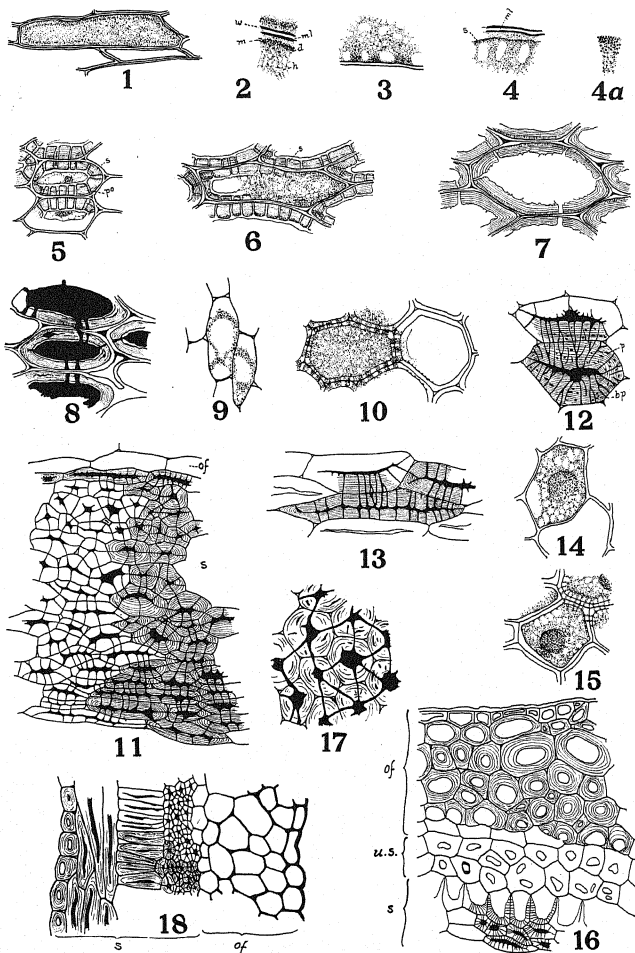
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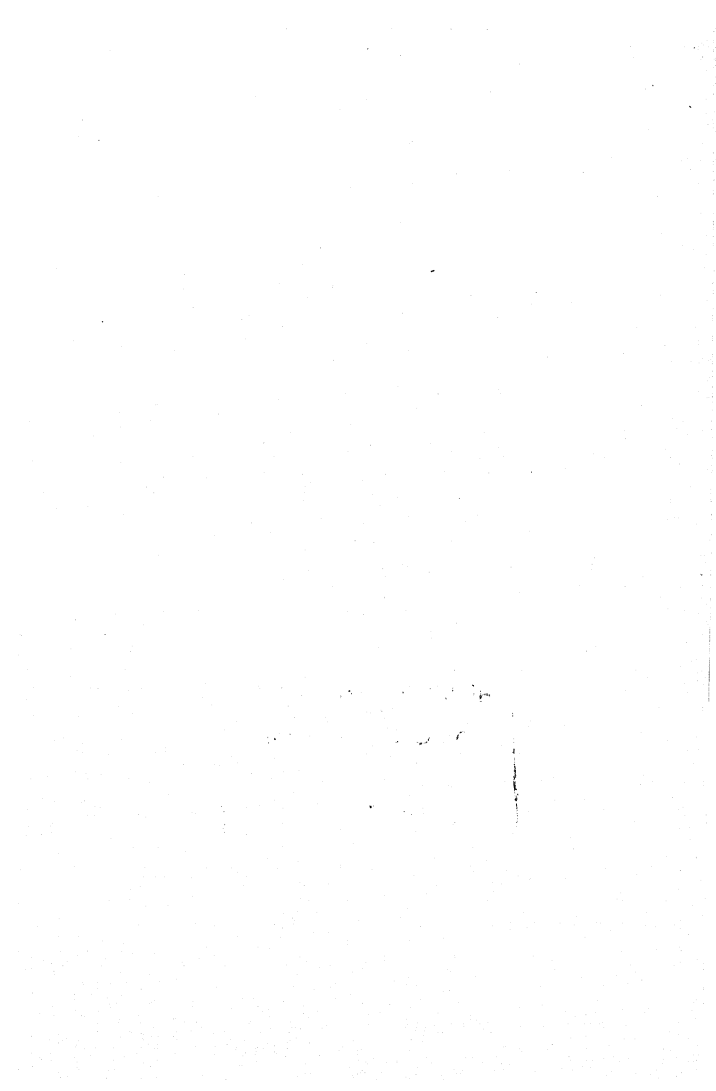
EXPLANATION OF PLATE XI

Abbreviations: *w*, walls of cells; *ml*, middle lamella; *m*, material piled in large granules here; *d*, dense portion; *h*, very small granules, homogeneous region, cytoplasm made up of fine granules arranged in rows; *s*, striations; *p*, pits; *po*, pores; *bp*, branched pores; *of*, outer fleshy layer; *us*, unsclerified cells; *s*, stony layer.

FIGS. 1-8.—*Ceratozamia mexicana*: fig. 1, cell from longitudinal section of ovule, showing early phases of pitting; piling of granular matter near walls evident; fig. 2, detail of portion of cell in fig. 1, with middle lamella, walls of cells, dense less granular cytoplasm, granules being piled toward walls, and vacuolation, $\times 627$; fig. 3, portion from much older cell, showing beginning of pitting, vacuoles increased in size, $\times 502$; fig. 4, later stage in pitting, with glimpse of first striation, from ovule older than that of fig. 3, $\times 502$; fig. 4a, portion of fig. 4, showing alignment of granules constituting area between vacuoles, $\times 627$; fig. 5, stone cells of outermost region of stony layer adjacent to outer fleshy layer, showing pores and striations of thick secondary walls, $\times 300$; fig. 6, stone cell from fifth row of cells within, counting from outer fleshy layer, showing striations in secondary wall, also vestiges of vacuolation,



QUISUMBING on STONY LAYER



×300; fig. 7, cell near inner fleshy layer with few pores and large lumina, and palisade striations of old wall, ×300; fig. 8, innermost cells of stony layer, showing persistence of tannin in lumina and also in pores; pits quite common in secondary walls, ×300.

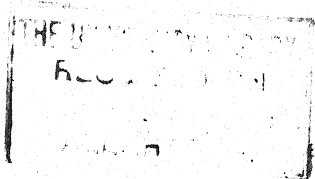
FIGS. 9-13.—*Ginkgo biloba*: fig. 9, two cells of fig. 38, showing arrangement of cytoplasmic material, ×502; fig. 10, cell in pitting stage, ×502; fig. 11, median longitudinal section of stony layer, showing different types of cells; elongated longitudinal cells on outside, isodiametric cells in middle, and elongated longitudinal cells in interior (more or less diagrammatic), ×166; fig. 12, stone cell in middle region, showing pits in secondary walls, branched forking pores, and striations, ×300; fig. 13, cells from innermost region of stony layer, showing elongated longitudinal type of cells, striations, and few branched pores, ×300.

FIGS. 14, 15.—*Pinus Laricio*: fig. 14, cell before lignification, showing prominence of vacuoles, cytoplasmic material, and ruptured nuclear membrane, ×502; fig. 15, cell from same ovule as fig. 14, taken near micropylar end, showing beginning of pitting stage; note dense spots near primary walls, ×502.

FIG. 16.—*P. Banksiana*: diagram of transverse section of ripe seed, showing portion of lignified rib and stony layer; outer fleshy layer shown with lignified rib and parenchymatous cells within; stony layer shows unsclerified region on outside, U-shaped cells of stony layer, isodiametric cells in inner region, ×260.

FIG. 17.—*Juniperus virginiana*: cells of old seed in transverse section, showing few canaliculi and broken striations in secondary walls, ×300.

FIG. 18.—*Gnetum Gnemon*: transverse section of outer integument, showing outer fleshy and stony layers, ×166.



PHYSIOLOGY AND MORPHOLOGY OF PYTHIOMORPHA GONAPODIOIDES¹

BESSIE B. KANOUSE

(WITH PLATES XII, XIII)

The important rôle of the environment in the morphological development of fungi is a fact too often overlooked or underestimated. That such an intimate relation exists between a fungus and its surroundings has been demonstrated beyond doubt. It is especially apparent in the Phycomycetes. In the Saprolegniaceae and in related families, the physiological relations can be predicted with great accuracy; yet even here it sometimes happens that due regard is not given to these significant facts, with the result that accounts of laboratory studies often give erroneous descriptions of the possible normal morphogenesis.

A striking example of the value of controlled cultures was evident in connection with some studies made on *Pythiomorpha gonapodioides* Peterson. The point of interest in connection with this fungus lies in the fact that it shows particularly delicate reactions to unfavorable external stimuli, in marked contrast to the reactions in what are probably optimum conditions. Hence there exists a disagreement in the accounts given for sporangial development by the two investigators who have previously reported the fungus. PETERSON (3) originally described the fungus from Danish collections, erected for it the new genus *Pythiomorpha*, and established the new family Pythiomorphiaceae. According to his description, the genus differs from *Pythium*, which it is said to resemble closely, in the absence of a vesicle membrane, which in *Pythium* is formed outside the sporangium, and in which the zoospores are completed. He says concerning *Pythiomorpha* that the zoospores "are not surrounded by a vesicle membrane, and are at once capable of movement." From observations based on mixed cultures, VON MINDEN (2) described the same fungus from some collections in Germany, and states that there is present a vesicle membrane similar to that found in *Pythium*;

¹ Papers from the Department of Botany of the University of Michigan, no. 215.

hence he believes that *Pythiomorpha* might quite reasonably be included in the Pythiaceae.

In the following account, the studies were made from cultures derived from single zoospore isolations of the species studied by these two men. As will be seen, PETERSON's account of the sporangial development was correct; VON MINDEN's misinterpretation was due doubtless to lack of a full appreciation of the effect of the physiological conditions in the development of this sensitive organism. The present account confirms the fact that *Pythiomorpha* cannot be placed in the Pythiaceae, if we accept the definition of that family as it was established by SCHRÖTER (5). This account extends the description of *Pythiomorpha gona-podioides*, since it is the first time that the sexual reproduction has been seen definitely. PETERSON never saw the sexual organs, and VON MINDEN's observations were made on mixed cultures in which the connections could not be established with any degree of certainty.

Reactions in culture

The fungus was obtained from two sources and a total of four collections was made. The collection upon which this account is based was obtained in November 1923, from a brook near Ann Arbor, Michigan. It was secured by using fruit bait in the manner described by VON MINDEN. After two weeks, when mycelium appeared on the surface of the fruit (peach, prune, and fruit of a *Crataegus* sp. were used), the traps were brought into the laboratory. The fruit was washed carefully and repeatedly in water, placed separately in glass capsules containing about 200 cc. distilled water, and put in a cold room. The temperature ranged from 2 to 14° C. Bacteria, Paramecia, etc., developed readily, and it was only by changing the water frequently that such contaminations could be kept at a minimum during the time the rough cultures were kept in the laboratory. There soon appeared sporangia characteristic of *Pythiomorpha*. Nearly all of them were poorly or only partially developed. In microscopic mounts no activity showed within the sporangia, and no motile zoospores were seen. Only one sporangium was observed which contained a few nonmotile spores. This served later in selecting *Pythiomorpha* spores for isolation. An effort was

first made to obtain mass cultures that would be comparatively free from contaminations, from which spores could be isolated more easily. After repeated failures in obtaining a growth from such fragments on the substrata as they were first used, this procedure was abandoned and single spores were isolated directly from the original mixed culture, using the Kauffman spray method.

Failure to secure gross cultures made from mycelium fragments on flies, cooked prune, uncooked prune, or beef gelatine discouraged the use of such material in the handling of the several single spore isolations that were made. It was discovered, however, that prune agar, synthetic agars with a high sugar content, or sugar gelatine were favorable for mycelial growth; so the stock cultures were made by transferring small blocks of these agars into flasks containing sterile conductivity water. The vegetative growth was luxuriant, but no sporangia were formed. The fungus also made rapid growth in pea broth, but here likewise no sporangia developed. Small portions of the mycelium were washed in conductivity water for two hours, and then were transferred to 15 cc. fresh conductivity water, following the method used for securing sporangia in *Saprolegnia*. This method of quickly and thoroughly reducing the food supply resulted in a production of sporangia after twenty-four hours. With few exceptions, however, the sporangial development had been checked before the spores were formed. The protoplasm was often plasmolized and contracted into an irregular mass in the center of a sporangium. Frequently the sporangiophore had resumed its apical growth, and after entering the sporangium grew around or through the protoplasmic mass (fig. 17). Often the protoplasm had entirely disintegrated, having been partially scattered outside the mouth of the sporangium (fig. 16). Some sporangia, chiefly those in an early stage of development, looked turgid and normal. In such cases vacuolization or evidences of cleavage could sometimes be seen clearly, but activity was suspended and spores were not developed. Fig 20 shows a sporangium in which germination of spores had begun within the sporangium. It was frequently observed that the tip of the sporangium broke and a part of the protoplasm flowed out in a formless mass and remained as such at the mouth of the sporangium (figs. 18, 19). The angular spore outlines could often be dis-

tinguished. Such sporangia are identical with those figured and described by VON MINDEN as normal *Pythiomorpha* sporangia. He interprets these sporangia to be like those found in *Pythium*, in which the protoplasmic contents is forced out of the sporangium into a vesicle formed at its opening.

In *Pythiomorpha gonapodioides*, what appears as a vesicle with spore contents is merely another form of abnormal sporangial development. In such a case the tip of the papilla opens, and the protoplasmic contents is extruded. It is probable that the inner membrane that develops before the spores are released is partially forced out of the sporangium, always at its apex, appearing somewhat like a vesicle. The protoplasm completely fills the membrane from its first appearance outside of the sporangium; it is never large enough to contain more than half of the sporangial contents. Furthermore, zoospores were never seen to form from such extruded protoplasm.

The last described sporangia, occurring as they do in single spore cultures under certain conditions, as well as in rough cultures grown in the laboratory, might quite readily be interpreted as normal sporangia. The predominance of sporangia with plasmolized and fragmented protoplasm, however, together with the absence of motile zoospores, indicated strongly that something was decidedly wrong with the environmental conditions; some inhibiting factors could be assumed as having prevented the fungus from developing sporangia and zoospores in the manner that is possible in nature, and inherent in the organism. Notwithstanding the fact that earlier efforts at using fruit as a culture medium had failed, it still seemed reasonable to infer that fruit in some form could be used successfully, since the original catches were made on this substratum. Further attempts resulted in the following method, which was used with decidedly satisfactory results.

The surface of a dry prune was cut away with a sterile scalpel. Small strips of the flesh, about 1 cm. in length and 4 or 5 mm. in width and of similar thickness, were dropped into hot paraffin. When cold a piece of prune was cut out, leaving a surrounding layer of paraffin. This prune fragment was then cut into two pieces, one of which was placed into a capsule containing 15 cc. of sterile conductivity water. By this method only a small cut surface about

5 mm. square was exposed directly to the water. The melted paraffin that entered the tissues of the prune together with the layer of hardened paraffin forming the partial external shell prevented a rapid diffusion of the soluble nutrients contained in the prune. The hot paraffin also killed any contaminations that might have been present, thus making it a simple matter to handle the single spore material and keep the cultures pure. Mycelium transferred to this substratum grew luxuriantly. After four or five days small portions of the well nourished mycelium were washed in conductivity water and then were transferred to fresh conductivity water. Within forty-eight hours an abundance of zoosporangia were formed, none of which showed the abnormal conditions just described. Zoosporangia were watched in all stages of development, and in numerous cases mature zoosporangia were seen to discharge their zoospores. The zoospores were "at once capable of movement" (3) and immediately swam away. This is the genus characteristic established by PETERSON, and is beyond question the correct account of zoospore formation under what must be assumed to be the optimum conditions for this fungus.

The details of sporangial formation were studied mainly from open slide cultures, since the disturbance caused by even a careful transfer of mycelium from a culture to a slide, made after the sporangia were formed, was sufficient to arrest more or less completely the activity within the sporangia. Mycelium was therefore transferred to conductivity water on flamed slides, which were placed in sterile Petri dishes made to serve as damp chambers. Fine glass threads were used to support the cover glass, since placing it directly on the mount interfered with sporangial activity. Van Tieghem cells were also used, but were not as satisfactory as the open slide cultures. Apparently the temperature best suited for sporangial development was about 22° C., so that all operations could be carried on in a room of ordinary temperature without much thought being given to that part of the technique. A temperature of 30°, likewise temperatures of 3° and 8° C., proved unfavorable; at these temperatures sporangia were formed tardily and presented the abnormal conditions before mentioned.

Oogonia were first seen in the rough cultures in the laboratory in

the fall soon after these cultures had had the thorough, frequent washings described. These oogonia probably belonged to *Pythiomorpha*, although their identity could not be established at that time. Oogonia were observed again on mycelium from single spore material in a conductivity water culture seventeen days old. Production of sporangia preceded the formation of oogonia. The third instance in which oogonia were seen was in an original rough culture that had been kept throughout the winter. An examination of this material was made in March, five months after its collection, when an abundance of germinating oogonia was observed. The question arose, could one be sure that these were oogonia of *Pythiomorpha*, or perhaps of another fungus? A single oogonium, therefore, was isolated and transferred to prune agar, from which source bacteria-free cultures were derived. After a period of time necessary to secure well nourished mycelium and to allow for the usual reactions to take place, mycelium was transferred to water in the usual way, and typical *Pythiomorpha* zoosporangia were formed. This proved that the oogonia seen in the rough culture belonged to *Pythiomorpha*, and the studies of the germinating oospores described later were therefore made from this culture.

The last two instances cited confirm the well known principle of KLEBS (1) that here, as in *Saprolegnia*, certain physiological conditions determine the appearance of the sex organs. The outstanding physiological condition in each of these cases was a gradual reduction of the suitable food supply from a mycelium which in the beginning had been in a vigorous, healthy condition. In the cases of the rough cultures in which the germinating oospores were found, the occasional washings throughout the winter, followed by a fresh supply of water, lessened the amount of available nutrients. Only a short time before the oospores were observed, the water in the culture had again been renewed. In the case of the mycelium from the single spore material placed in conductivity water, the same principle held true. In both cases the fungus responded by the production of sexual organs.

***Pythiomorpha gonapodioides* under optimum conditions**

The mycelium is very delicate as to size and fragility. It forms a loose, floccose mass of hyphae having a silvery white sheen. The

hyphae branch profusely in a subdichotomous manner (fig. 14). Budlike projections (fig. 14a) are present on all the mycelium, except on the hyphae bearing the reproductive organs. These outgrowths, which appear to be merely vegetative in nature, give the mycelium a very uneven appearance, hence a single hypha varies greatly in width, measuring 4-7 μ . Cross walls form only in hyphae immediately below the reproductive organs. The protoplasm is largely homogeneous in structure (fig. 15), producing a high refraction and a pallid color under high magnification. Granules which PETERSON believes are cellulin are always present. The hyphae occasionally form curiously knotted and twisted mats (fig. 21) that resemble the hyphae of certain *Phytophthora* species.

The long, straight, slender, unbranched sporangiophore hyphae (fig. 14b) extend beyond the central mass of branched mycelium, giving the margin of the culture the appearance of being bordered by a very delicate fringe. They are much narrower than the vegetative hyphae, averaging only 3 μ in width. The thin walled young sporangia form at the ends of the sporangiophores. They are at first spherical (fig. 1), but soon after the laying down of the basal septum they take on the characteristic oval or pyriform shape (figs. 2-4). There is a broad papilla at the apex of each sporangium (fig. 4). The fine, granular protoplasm of the sporangium is soon transformed into spores in a manner similar to that described by ROTHERT (4) for *Saprolegnia*. The protoplasm soon becomes coarsely granular, producing a lumpy appearance. Hyaline cleavage furrows appear from the center of the sporangium, dividing the protoplasm into about seven irregular shaped masses. Furrows continue to cut into these masses very quickly, until what are presumably the final spore units can be distinguished (fig. 3). The sporangium is divided by cleavage, so that in median section three or four rows of spores are seen across the short diameter of the sporangium (fig. 3). The vacuoles which are present in the spore origins shift constantly. The stage of angular spore units is followed by one in which the protoplasm appears more or less fused (fig. 4). The individual spores reappear very quickly, however, and at this time have more rounded outlines (fig. 5). A large vacuole and seven or more highly refractive globules as well as granular protoplasm can be seen within the

spores lying near the upper surface of the sporangium. Immediately before the spores are discharged, their outlines merge again (fig. 6). This lasts only a few seconds, when the sporangium is opened and the spores escape (fig. 7). At maturity the sporangia measure $26-48 \times 16-27 \mu$.

During the development of the zoosporangium, up to the time of zoospore discharge, considerable changes are taking place in the character of the sporangial wall. In the young sporangium the wall is thin, but as development continues differentiation occurs, as may be seen by comparing figs. 1-7. The wall as a whole increases in thickness, and is finally seen to be differentiated into an outer and an inner wall, between which a hyaline layer has become noticeable. Correlated with this "zwischen substanz" we find what appears to be the same kind of substance, making up the enlarged area produced during the development of the papilla at the tip of the sporangium. As shown in fig. 6, this substance disappears at the time of the opening of the sporangium, thus making free egress of the zoospores possible. The nature of the wall was determined in normal sporangia and mycelium in part by the following test. When stained with chloriodide of zinc the hyphal walls turn a pale lilac blue, indicating the presence of fungus cellulose. The outer zoosporangial wall also assumes a similar color. Furthermore, the inner membrane of the zoosporangium shows unmistakably the same color reaction; hence the inner membrane can be regarded as a definite, differentiated wall.

In the later stage of maturity, the space between the outer wall and the inner membrane at the apex of the sporangium becomes very clear (fig. 5). When the substance in the papilla has disappeared, the outer wall is spread by the break, so that the opening is frequently considerably wider than the diameter of the escaping zoospores (figs. 6, 7). The inner wall is unbroken until the discharge of the spores ruptures it at a point just beneath the papilla. That the opening made in this inner wall or membrane is small, is shown by the fact that the spores are very much constricted in passing through it (fig. 7). As soon as the spores leave the sporangium, the inner wall contracts and draws away from the outer wall, so that it is always visible in empty sporangia (figs. 9, 13). It

may be lifted by a rejuvenating sporangiophore growing into the empty sporangium at the base (figs. 9, 11).

The sporangia are true zoosporangia, since they never have been seen to function as conidia in the production of germ tubes. The number of zoospores formed within a zoosporangium varies from eight to thirty; they measure $9-15\ \mu$ in diameter. As soon as the zoospores are seen to move within a sporangium they show great activity. The plastic cells resolve themselves into curious shapes before they finally swim away. Their adjustment to conditions outside of the sporangium takes but a few seconds, so that the more or less angular form is rapidly changed to the usual kidney shape common to zoospores in the Peronosporales. The large vacuole finally occupies a position near the middle of the motile spore (fig. 8). There are two lateral cilia which can be seen clearly by staining with iodine vapor (fig. 8a). They were also observed three times in the living state. After a few rotations on the long axis, the zoospores swim away with long, swift, straight darts. On coming to rest the cell rounds up and encysts within a thin wall (fig. 8b). Germination may take place directly within a few hours by sending out hyphae in one or more places (fig. 8b). A second swarming then occurs, in which the protoplasm passes slowly out of the cyst wall (fig. 8c). The cilia were not located at this stage, but their escape from the cysts was clearly seen (fig. 8d).

Secondary sporangia are numerous. They are always formed at the end of a sporangiophore, either within an empty sporangium or at some distance beyond it (figs. 10-13). The process of the rejuvenation of the apex of the sporangiophore with the formation of new sporangia may be repeated several times, so that it is not uncommon to find a series of four or five empty sporangia in a row, or to find the same number arranged in a nested fashion (figs. 12, 13). Their development is in every respect like that given for the primary sporangia.

Oogonia are produced on short mycelial branches (figs. 22, 24). A cross wall separates the organ from the supporting hyphae. The oogonia are spherical in shape and measure $22-36\ \mu$. At maturity they are surrounded by three walls (fig. 24). The protoplasm is highly refractive, very fine, and evenly distributed, completely filling the

oogonium (figs. 23, 24). Many oogonia develop parthenogenetically. On germination the slender hyphae which are put out usually branch immediately (figs. 26-28). In the hundreds of germinating oogonia observed no case was seen, under the conditions obtaining, where sporangial formation directly followed germination.

Antheridia were infrequent in the cultures under the conditions named. Only about 10 per cent of the oogonia were accompanied by antheridia. They were seen only in the single spore cultures, since, as before stated, the oogonia in the mass culture were already germinating when first observed. So far as could be determined, the antheridial branch arises from a hypha other than that bearing the oogonium. The antheridial branch winds around the oogonium, as shown in fig. 25. A cross wall separates the antheridium from the supporting hypha on which it is formed.

This work was done under the direction of Professor C. H. KAUFFMAN, University of Michigan, to whom I wish to express my thanks for kind advice and help.

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EXPLANATION OF PLATES XII, XIII

FIGS. 1-15 from single spore cultures developed under optimum conditions; all excepting fig. 14 drawn to scale from high power.

FIG. 1.—Young zoosporangium and sporangiphore.

FIG. 2.—Zoosporangium at later stage with papilla.

FIG. 3.—Zoosporangium with cleavage furrows separating what are probably final spore origins.

FIG. 4.—Later stage of development, showing indistinct spore outlines.

FIG. 5.—Next stage, showing distinct rounded outlines of individual spores.

FIG. 6.—Appearance of zoospores immediately before zoosporangium opens; outer wall as it occasionally looks immediately preceding escape of spores.

FIG. 7.—Egress of zoospores and manner of passing through opening.

FIG. 8.—Biciliated zoospore of first swarm stage, soon after escaping from sporangium: *a*, immediately after coming to rest (stained with iodine); *b*, encysted spore and germination; *c*, naked cells during and after escaping from cyst; *d*, germination of such a naked cell by formation of germ tube.

FIG. 9.—Empty zoosporangium, showing inner membrane lifted by tip of rejuvenated sporangiophore pushing into base.

FIG. 10.—“Nested” appearance of secondary empty sporangia, and early stage of fourth secondary zoosporangium.

FIG. 11.—Later development of secondary zoosporangium (note rising of inner wall at base of empty sporangium).

FIG. 12.—Nest of four empty sporangia with stalk of fifth; note that at formation of fifth sporangium a sufficient amount of growth energy had in all probability again been provided by some sudden and favorable change in food supply.

FIG. 13.—Series of empty zoosporangia formed within and beyond primary zoosporangium; sixth beginning to develop.

FIG. 14.—Habit sketch of mycelium grown in prune culture five days, washed two hours, transferred to conductivity water twenty-four hours: *a*, vegetative, budlike protuberances; *b*, sporangiophore in relation to branched mycelium.

FIG. 15.—Detail of hyphae enlarged.

FIGS. 16-20.—Various types of abnormal sporangial development, showing effects of unfavorable conditions; single spore cultures; drawn to scale (high power).

FIGS. 16, 17.—Contents of sporangium in plasmolyzed condition.

FIGS. 18, 19.—Protoplasm extruded from sporangia, giving false impression of a vesicle.

FIG. 20.—Spore germination in situ.

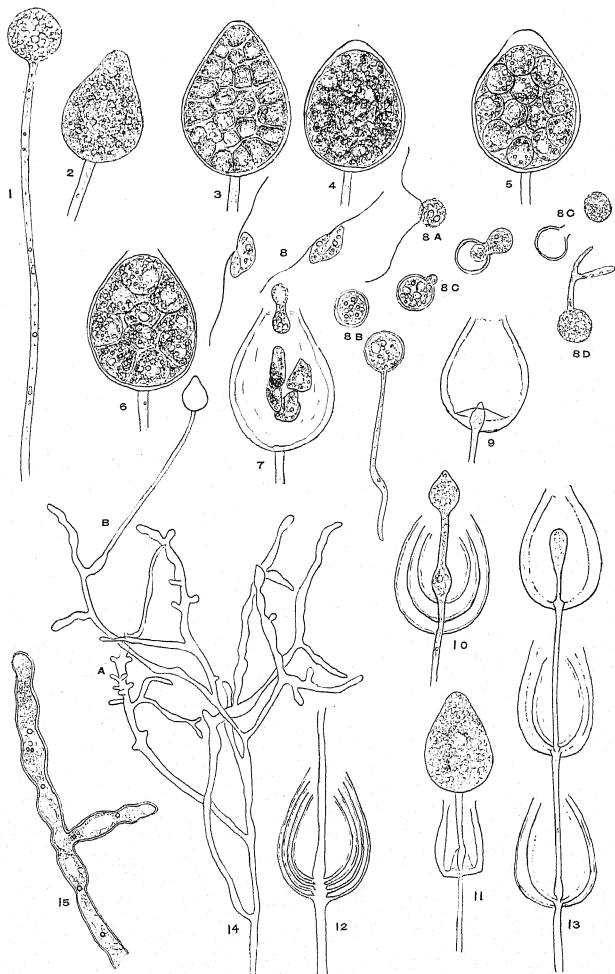
FIG. 21.—Knotted type of mycelium produced by growth of budlike projections (found in all old cultures).

FIGS. 22, 23.—Early stages in development of normal oogonia.

FIG. 24.—Mature parthogenetic oospore and oogonium.

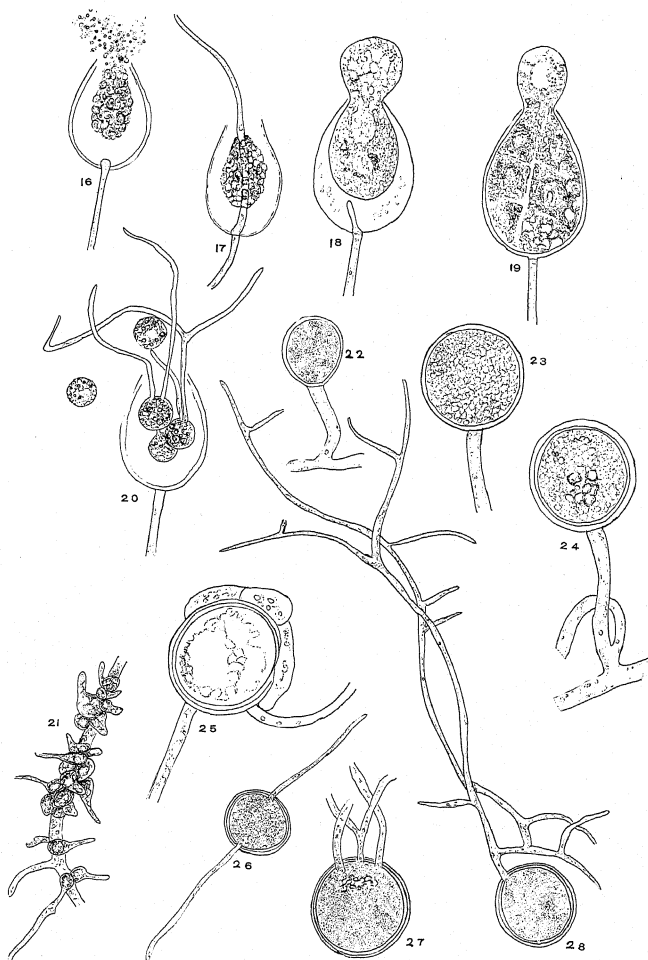
FIG. 25.—Oogonium with antheridium.

FIGS. 26-28.—Germinating oospores from culture, shown by isolation to be those of *Pythiomorpha*.

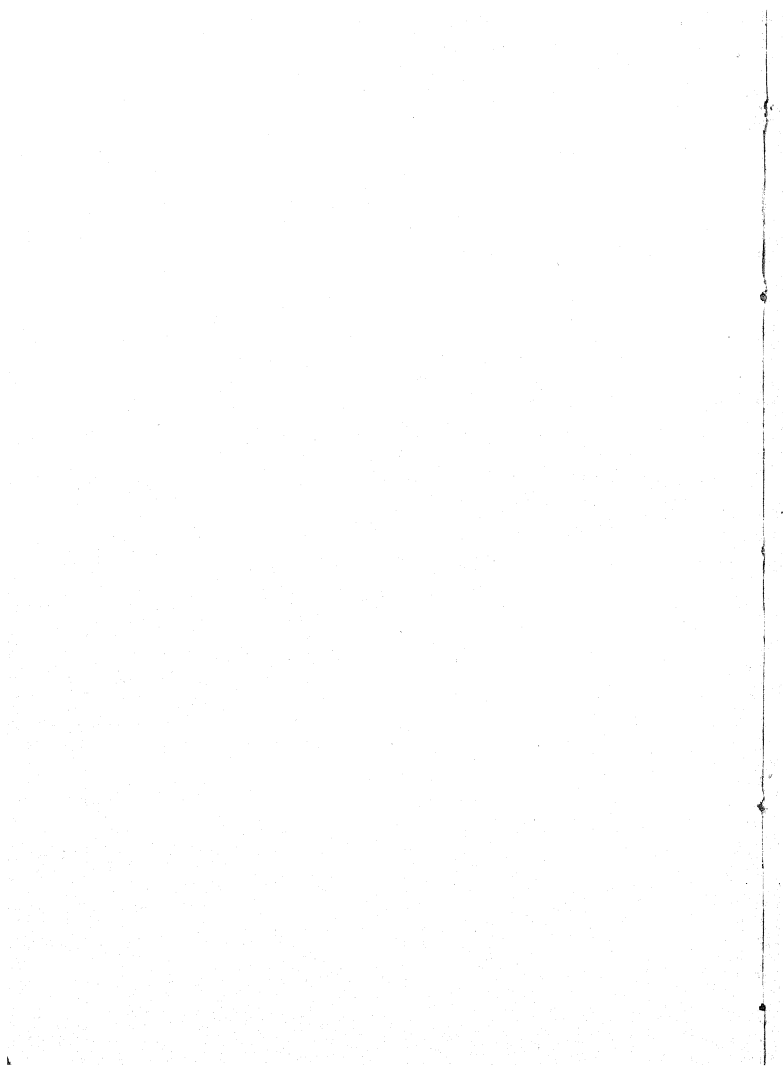


KANOUSE on PYTHIOMORPHA





KANOUSE on PYTHIOMORPHA



DEVELOPMENT OF EMBRYO SAC IN GASTERIA, CYRTANTHUS, AND VELTHEMIA

ETHEL G. STIFFLER

(WITH PLATES XIV, XV, AND SEVEN FIGURES)

Introduction

The study of the female gametophyte in angiosperms began during the latter half of the nineteenth century, and developed chiefly through the efforts of such investigators as HOFMEISTER, WARMING, VESQUE, STRASBURGER, TREUB, MELLINK, GUIGNARD, and others. COULTER and CHAMBERLAIN (2) summarized the work which had been accomplished up to 1903, and recently another summary by RUTGERS (13) has appeared. On the whole, the subject has received a much smaller proportion of scientific attention during this century than formerly.

The history of the female gametophyte is considered as beginning with the division of the megaspore mother cell. Two general types of development prevail. The first, or so-called "normal" type, is that in which the usual product of the megaspore mother cell divisions is an axial row of four megaspores. With but few exceptions, the three outer cells disintegrate, while the innermost, by three successive divisions, gives rise to the mature 8-nucleate embryo sac. In the second, known as the "*Lilium*" type, the megaspore mother cell develops directly into the 8-nucleate embryo sac, without the intervention of megaspore formation. There is every gradation between the two types. The variety is quite conspicuous among the Liliaceous plants, due perhaps, as suggested by COULTER and CHAMBERLAIN, to the fact that more of the species have been investigated. These authors felt safe in making only one generalization in regard to the monocotyledons, which was that, aside from their irregularities, more of them than of the dicotyledons had reached the condition of the non-dividing mother cell. The present study will include only conditions found among the Liliaceae, Amaryllidaceae, and Iridaceae.

Review

The division of the megaspore mother cell may result in the formation of four, three, or two megaspores, or the cell itself may develop, by three divisions, into the complete embryo sac. Walls may or may not form between the cells, and if formed may be horizontal, vertical, or oblique, altering to some degree the shape and arrangement of the megaspores. They may be evanescent, lasting only a short time, or very definite walls which survive the degeneration of the megaspores. Such variations, however, have no effect upon the morphological position of the megaspores. The first division of the megaspore mother cell is always a reduction division, while the second is equational.

If the megaspore mother cell divides, it is usually the inner daughter cell which develops, the other disintegrating. There are, however, exceptions to this rule. In *Smilacina racemosa* (9), and probably also *S. amplexicaulis*, the megaspore mother cell divides unequally to form a large outer and a small inner cell. The outer becomes the embryo sac, while the inner produces two cells which gradually degenerate. In *Scilla* (*Agraphis*) *patula*, *S. nutans* (18), *S. campanulata*, and *S. hyacinthoides* var. *caerulea* (11) the outer cell develops, although in the last two species the inner also develops to the 4-nucleate stage before degenerating. (The usual development of the inner cell, as well as the fact that it usually starts division first, possibly may be accounted for by the fact that it is nearer the source of nutriment through the base of the ovule.)

The normal and *Lilium* types of development have been outlined and will be described in detail in connection with *Gasteria* and *Cyrtanthus*. It seems worth while, however, to mention some of the reported cases of these two general types. To the normal type belongs *Polygonatum commutatum*. COULTER and CHAMBERLAIN reported *Trillium recurvatum* as developing in a similar manner, but Miss HEATLEY (7), after working out the life history of *T. cernuum*, in which the embryo sac forms from the two inner megaspores, came to the conclusion that these authors were mistaken, and that *T. recurvatum* was like *T. cernuum*. Another example of this type, *Convallaria majalis*, has also been a subject of discussion. It was investigated by WIEGAND (2), who reported that the megaspore mother

cell divides to form two fully separated daughter cells, which in turn divide without forming cross walls. Then the transverse wall disintegrates and the four nuclei divide to form the 8-nucleate sac. This account was contradicted by SCHNIEWIND-THIES (16), who reported that the mother cell divides to form a linear tetrad, one cell of which develops while the others degenerate.

Examples of the *Lilium* type are *Lilium Martagon* (12, 14), *L. philadelphicum* (15), and *Tulipa* (18). Miss FARRELL (4), working on the vascular anatomy of *Cyrtanthus sanguineus*, mentioned the probable conformity of its embryo sac to this type. *Smilacina stellata*, *S. sessifolia*, *Maianthemum canadense*, and *Medeola virginica* (9) represent a somewhat intermediate stage. In these tetrad formation occurs, the cells being separated by walls which later dissolve and disappear. Then by one division the embryo sac is completed.

Further departures from the two general types are those in which only two daughter cells are formed, several of which have already been described, or in which there are three. In both cases, the inner usually is the functioning cell which becomes the embryo sac. Plants developing the diad are *Streptopus roseus* (9), *Paris quadrifolia* (in which the outer nucleus also may divide once), *Trillium grandiflorum* (3), *Narcissus tazetta*, *N. micranthus*, and *Ornithogalum pyrenaicum* (6). Three daughter cells are found in *Yucca gloriosa* and *Iris stylosa* (3).

A type of embryo sac development rare for Liliaceous plants is that of *Clintonia borealis* (17). Here the archesporial cell undergoes no cell division, neither cutting off a parietal cell nor dividing into megaspores, but passes directly into the embryo sac. After each maturation division, temporary cell plates are visible and the outer daughter nucleus is the larger. The outermost nucleus divides twice to form a mature sac of four nuclei. One peculiarity of this development is the unipolarity of the embryo sac after reaching the tetrad stage. Another is that only four nuclei are produced, and that these resemble the micropylar four nuclei of the normal 8-nucleate sac. The two outer nuclei become synergids, the others the egg and one polar nucleus. McALLISTER (9) confirmed these results.

Abnormalities, particularly in the form of two megaspore mother cells, have been reported, but seem to have occurred with

greater frequency among the dicotyledons. Among the monocotyledons the earliest case on record is that of *Ornithogalum pyrenaicum*, in which GUIGNARD (6) figured and interpreted two small hypodermal cells as being archesporial. BERNARD (1) reported two embryo sacs in the same nucellus in *Lilium candidum*. COULTER and CHAMBERLAIN noted two cases in *Lilium philadelphicum*, one showing three and the other five archesporial cells. Miss FERGUSON (5) discovered a single case of two megaspore mother cells separated by a layer of somatic cells in a megasporangium of *Lilium longiflorum*. LECHMERE (8) reported two embryo sac mother cells in one ovule of *Fritillaria messanensis*. McALLISTER (9), working on the Convallariaceae, found two megaspores or partly developed embryo sacs in *Smilacina stellata*, *S. sessifolia*, *S. racemosa*, *S. amplexicaulis*, *Polygonatum commutatum*, and *Maianthemum canadense*. He attaches little significance to their occurrence. VESQUE (19) found an interesting case in *Uvularia grandiflora*, where two or three daughter cells of one megaspore mother cell could each develop an embryo sac to the 4-nucleate stage.

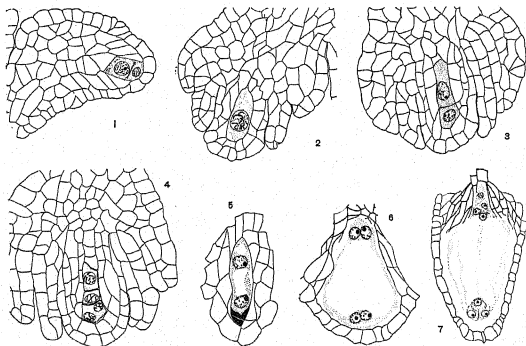
Material and observations

Gasteria, *Vellhemia*, and *Cyrtanthus*, the plants furnishing the material for this study, are all natives of South Africa. The first two belong to the Liliaceae, the last to the Amaryllidaceae. The ovaries of *Gasteria* and *Cyrtanthus* are very similar in structure, consisting of three carpels, bearing two rows of about fourteen ovules each. In *Vellhemia* only two ovules develop on each carpel, and it commonly happens that only four ovules of the six possibilities reach maturity. The ovaries were collected at various stages of development, and fixed in a chromosomo-acetic solution. Paraffin sections were cut 8-10 μ thick and stained in Haidenhain's haematoxylin.

GASTERIA

In the ovule of *Gasteria*, the one celled archesporium, distinguished by its size and position from neighboring cells, appears before the formation of integuments has begun. Division takes place shortly, resulting in a primary parietal or tapetal cell and the larger megaspore mother cell (text fig. 1). The former may degenerate almost immediately, or persist through the heterotypic divisions.

Meanwhile both the outer and inner integuments have made their appearance and are developing rapidly. The megaspore mother cell enlarges and elongates, the nucleus remaining at or near the center (text fig. 2). The ensuing division results in the formation of a large inner and a smaller outer daughter cell, separated by a definite wall (text fig. 3). The homotypic division follows, beginning first in the inner cell, and giving rise to a tetrad of four megaspores



FIGS. 1-7.—*Gasteria* ovules showing: fig. 1, megaspore mother and primary parietal cells, $\times 270$; fig. 2, megaspore mother cell, later stage, $\times 270$; fig. 3, diad stage, $\times 267$; fig. 4, tetrad, $\times 270$; fig. 5, 2-nucleate embryo sac with three disintegrating megaspores, $\times 270$; fig. 6, 4-nucleate embryo sac, $\times 200$; fig. 7, mature embryo sac, $\times 135$.

(text fig. 4). The wall which separates the two outer cells is typically oblique, although it may be horizontal and parallel to the other two. It forms rather slowly, and occasionally fails to appear before the nuclei have badly disintegrated.

The three outer megaspores degenerate as the fourth continues its development. The product of the first gametophyte division is a 2-nucleate embryo sac (text fig. 5). The three disintegrating megaspores form a cap at the micropylar end. The embryo sac grows, and after another division shows two nuclei at each end, separated

by the large vacuole which appeared in the preceding stage (text fig. 6). By this time the sac has broadened considerably at its micropylar end. Growth continues, division takes place a third time, and the 8-nucleate embryo sac is complete. Immediately after this division, the two polar nuclei migrate toward each other, meet near the antipodal group, and fuse. After a period of rest, the egg apparatus is organized from the two synergids and the egg nucleus at the micropylar end (text fig. 7). Often the antipodals have started to degenerate by the time the egg is ready for fertilization. No abnormalities of development were noted.

CYRTANTHUS

At the time when the large megaspore mother cell of *Cyrtanthus parviflorus* makes its appearance, the ovules are horizontal, with the inner integuments in the early stage of formation. A single layer of cells separates the megaspore mother cell from the epidermis. No tapetal cell is cut off. The growth of the mother cell is accompanied by that of the integuments, and by the time the heterotypic metaphase is reached the inner integuments have grown out the length of the nucellus, the outer ones half as far. The product of the heterotypic division is the 2-nucleate embryo sac with a large vacuole in the center between the nuclei (fig. 3). At this stage the growing ovules almost fill the cavity of the ovary. Continued growth results in the upward pushing of the ovules, so that the uppermost become almost vertical. The ovules in adjacent rows turn slightly outward from each other, resulting in a compression of the outer integument of one side. During subsequent growth, the embryo sac becomes broader at the micropylar end. The homotypic division forms the 4-nucleate stage, with two nuclei occupying each end of the sac (fig. 4). Often the antipodal nucleus has divided in a plane at right angles to the micropylar nucleus, resulting in one chalazal nucleus above the other. Preceding the third division, considerable growth and lengthening of the embryo sac takes place. The nuclei divide to form eight, thus completing the embryo sac. The outer polar nucleus moves toward the inner and they meet near the antipodal group (fig. 5). The three antipodals and the fusing polar nuclei continue to occupy the inner end of the sac, while at the outer end the egg

apparatus is organized (fig. 6). At this stage the outer portions of the synergids show striations.

Several abnormal stages were observed. Cases of two megaspore mother cells are shown in figs. 7 and 8. Another was found which resembled the twin megaspore mother cells of *Velthemia* (fig. 11). One example of four archesporial cells was found (fig. 9).

VELTHEMIA

The integuments of the comparatively large ovule of *Velthemia viridifolia* are well developed before there is any perceptible differentiation of the nucellar tissue to form the megaspore mother cell. It finally appears as a large cell in usually the second layer below the epidermis. No tapetal cell is cut off. The heterotypic division forms two daughter cells, the inner larger and separated from the other by a wall. The subsequent homotypic division results in the formation of a tetrad, of which the two inner megaspores are separated by a horizontal wall, the two outer by a vertical wall (fig. 14). The three outer megaspores rapidly disintegrate, while the inner continues its development (fig. 14). The 2-nucleate embryo sac has the nuclei as opposite ends, separated by a large vacuole (fig. 16), and by another division the 4-nucleate sac develops (fig. 17). Here normal development ceased in the material available.

Gasteria seemed to be normal in all respects, and *Cyrtanthus* showed few variations, but *Velthemia*, in addition to displaying many abnormal tendencies in early stages, seemed to be wholly abnormal in its later development. A double ovule was observed in one instance (fig. 10). Two megaspore mother cells developing side by side in the same nucellus are shown in fig. 11. In both examples the cells seemed entirely normal and capable of further development. An interesting case showed indications that a linear tetrad was formed, in which the three outer megaspores failed to degenerate and all four started to develop (figs. 18, 19). The two inner cells have reached the 2-nucleate stage, while the outer two are still uninucleate (fig. 18). A later stage is shown in fig. 19, where the nuclei of all cells have undergone division. The innermost megaspore has developed into the 4-nucleate embryo sac; the next one, also in the 4-nucleate stage, is being crushed from above and below; the third has become

binucleate but also is being crowded; the fourth has had room to expand and has also reached the 2-nucleate stage (fig. 19). A case somewhat similar to this is that reported and figured for *Uvularia* by VESQUE (19), in which two or three megaspores develop as far as the 4-nucleate stage.

Typical older embryo sacs are shown in figs. 20 and 21. The sac consists of two arms, one exceeding the other in length. By this time the ovule has grown quite long, and the longer arm of the sac extends well into it. In practically every case there is a very large nucleus near the tip of the long arm, and three, apparently the egg apparatus, at the micropylar end (fig. 20). Above the large chalazal nucleus numerous dark-staining granules are seen, possibly remains of other nuclei, and in the short arm two dark masses resembling degenerating nuclei of other preparations are seen in the embryo sac illustrated in fig. 21. Four nuclei occupy the micropylar end. In this preparation the sac consists of two parts joined by a median connection; the sac shown in fig. 20 is apparently a continuous structure.

A different type of development is shown in fig. 22. Here there are probably two rows of three megaspores each, the innermost of each group appearing more capable of development.

Conclusions

1. In *Gasteria* the archesporial cell forms the primary parietal and megaspore mother cells. The latter divides to form a tetrad of megaspores, the three outer of which disintegrate, while the inner, by three successive nuclear divisions, develops into the 8-nucleate embryo sac.

2. *Cyrtanthus* follows the *Lilium* type of embryo formation, the 8-nucleate sac developing directly by three nuclear divisions from the megaspore mother cell.

3. The megaspore mother cell of *Veltheimia* forms a tetrad of which only the innermost megaspore develops. The embryo sac develops abnormally beyond the 4-nucleate stage.

I wish to express to Dr. WILLIAM RANDOLPH TAYLOR, who suggested the study, my great appreciation of his kindness and cooperation in the preparation of this paper.

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EXPLANATION OF PLATES XIV, XV

All plate figures, with the exception of fig. 10, are so oriented that the micropylar end is toward the bottom of the page, and are longitudinal sections of ovules.

PLATE XIV

Cyrtanthus parviflorus

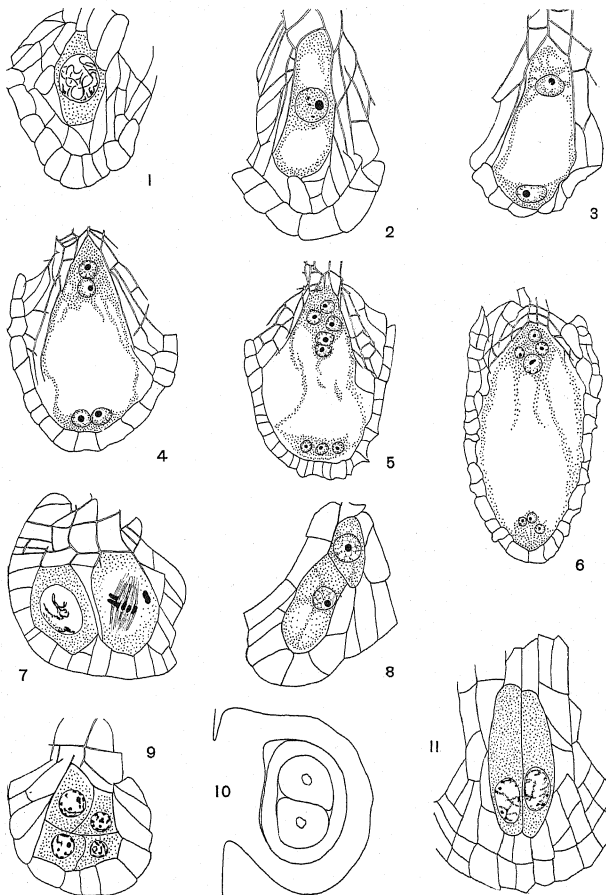
- FIG. 1.—Megaspore mother cell; $\times 400$.
FIG. 2.—Megaspore mother cell, later stage; $\times 400$.
FIG. 3.—Two-nucleate embryo sac; $\times 400$.
FIG. 4.—Four-nucleate embryo sac; $\times 300$.
FIG. 5.—Eight-nucleate embryo sac; $\times 200$.
FIG. 6.—Mature embryo sac; $\times 135$.
FIGS. 7, 8.—Two megaspore mother cells; $\times 400$.
FIG. 9.—Four megaspore mother cells; $\times 400$.

Vellthemia viridifolia

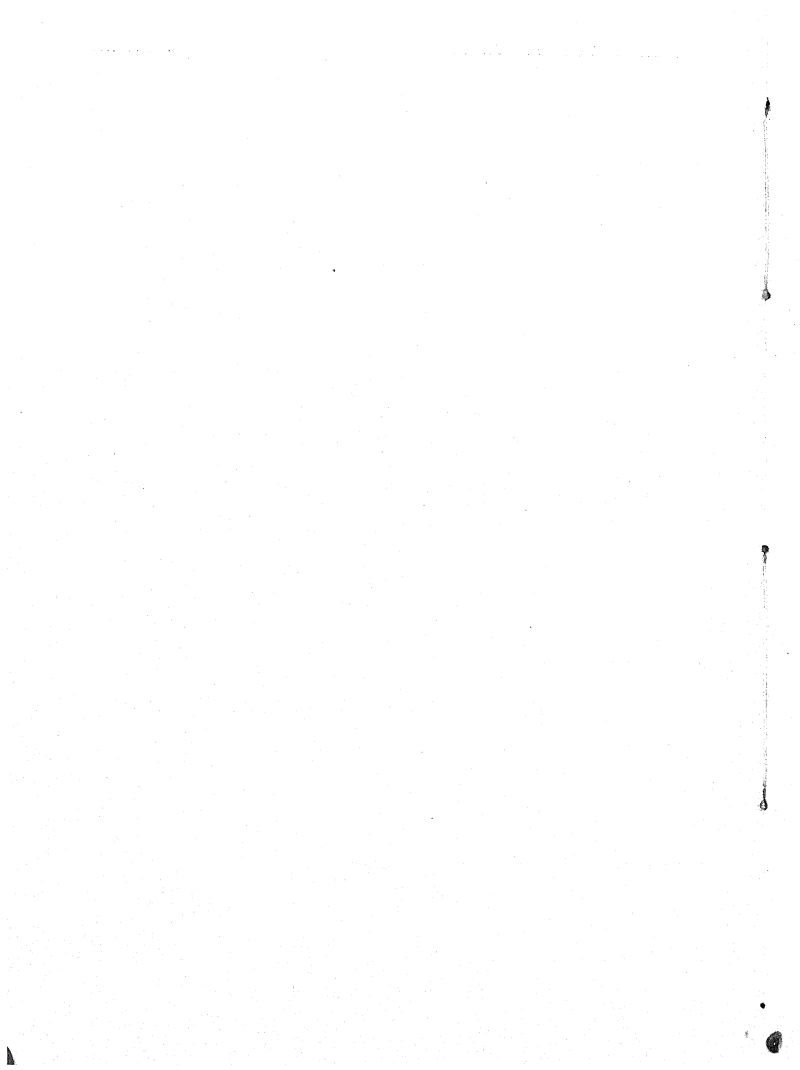
- FIG. 10.—Twin ovules; $\times 375$.
FIG. 11.—Twin megaspore mother cells; $\times 400$.

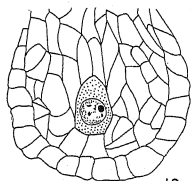
PLATE XV

- FIG. 12.—Megaspore mother cell; $\times 350$.
FIG. 13.—Diad stage; $\times 350$.
FIG. 14.—Tetrad; $\times 350$.
FIG. 15.—Developing megaspore; $\times 350$.
FIG. 16.—Two-nucleate embryo sac; $\times 350$.
FIG. 17.—Four-nucleate embryo sac; $\times 350$.
FIG. 18.—Four developing megaspores; $\times 350$.
FIG. 19.—Same, later stage; $\times 270$.
FIG. 20.—Abnormal late embryo sac development; $\times 135$.
FIG. 21.—Same; $\times 290$.
FIG. 22.—Two rows of three megaspores each; $\times 350$.

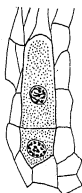


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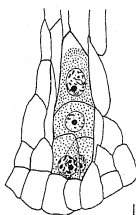




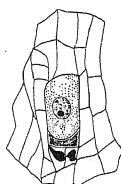
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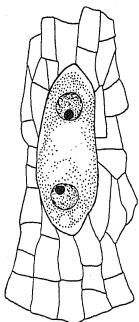
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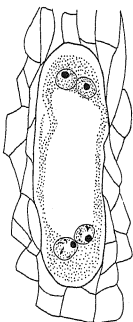
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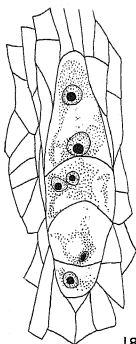
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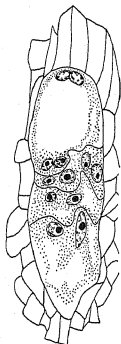
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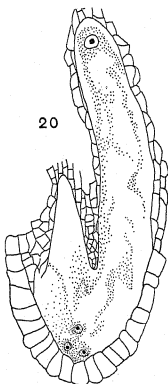
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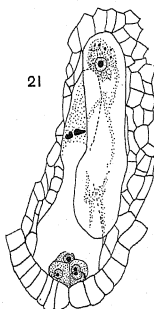
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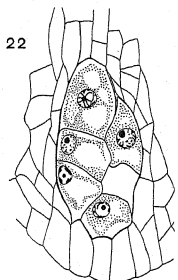
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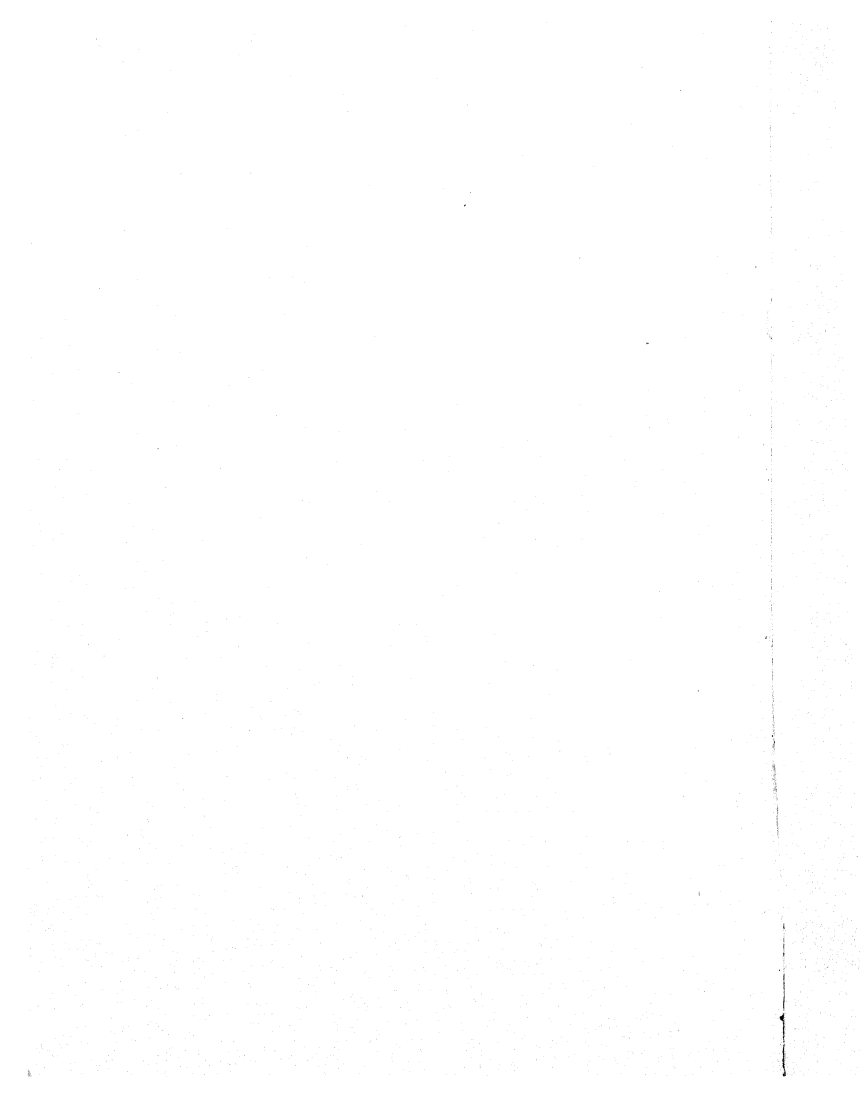
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FEMALE GAMETOPHYTE OF TRILLIUM SESSILE

R. C. SPANGLER

(WITH PLATES XVI, XVII)

Although some species of *Trillium* have a wide distribution and can easily be obtained in abundance, they have not been studied very extensively. Descriptions of the development of the female gametophytes of *T. grandiflorum* (2) and *T. cernuum* (4) have been published. GREGOIRE and WYGAERTS (3) used these same species for studying the structure of the nucleus and chromosomes. ATKINSON (1) studied microsporogenesis, and Miss NOTHNAGEL (5) fecundation and formation of the primary endosperm nucleus in *T. grandiflorum*. In studying the development of the female gametophyte of *T. sessile*, some attention has been given to the structure of the chromosomes at certain stages. A complete study, however, of the structure of the chromosomes and nucleus was not attempted.

T. sessile grows in abundance near West Virginia University. The material was collected from October 1 to 10, and during April and May. Flemming's weaker solution of chromo-acetic acid was used as the fixing agent. Haidenhain's iron-alum haematoxylin, and safranin and gentian violet were the stains used. The former gave better results.

Material collected October 1 showed that the archesporial cell had already divided to form the megaspore mother cell and a primary wall cell (fig. 1). In the formation of primary parietal tissue *T. sessile* is similar to *T. cernuum* (4) and unlike *T. grandiflorum* (2). At this stage the megaspore mother cell and its nucleus are much larger than the cells and nuclei of the surrounding tissue (fig. 1). Material collected in early spring showed no change, except that the megaspore mother cell was much larger (fig. 2). The nucleus shows the first indications of division two or three days before the spreading of the petals. The resting reticulum forms very thin threads (fig. 2), which collect at one side of the nucleus into a dense synaptic mass (fig. 3). As the threads come out of synapsis they shorten, thicken,

and segment into six chromosomes (fig. 4). At this stage, apparently just before the disintegration of the nuclear membrane, the chromosomes contain many vacuoles (fig. 5). A longitudinal section sometimes shows a row of vacuoles with equal spaces between them (fig. 5a). Cross-sections of chromosomes show different arrangements and sizes of vacuoles. Some have one large central vacuole, with or without smaller vacuoles around it (figs. 5b, 5c); others have four large vacuoles (fig. 5d). The nuclear membrane disintegrates and the chromosomes are quickly arranged in the equatorial region of the spindle (fig. 6). At this stage the chromosomes contain vacuoles (figs. 7, 7a) as well as immediately after the splitting (figs. 8, 8a). The spindle fibers are very numerous and strongly developed. This is quite different from *T. cernuum*, in which Miss HEATLEY observed that only "delicate spindle fibers can be distinguished in the cytoplasm."

At late anaphase each chromosome contained a row of distinct vacuoles with equal spaces between them (figs. 9, 9a). GREGOIRE and WYGAERTS observed a similar condition in the vegetative cells of *T. grandiflorum* at both metaphase and anaphase. These vacuolate chromosomes collect in a close mass at the poles and become invested with a membrane. The nucleus then enlarges; the chromosomes separate and show the same vacuolate structure as at late anaphase (figs. 10, 10a). At this stage in *T. grandiflorum*, ERNST reports that the chromosomes form a netlike reticulum as they come out of the close knot of early telophase. In *T. cernuum* Miss HEATLEY states: "The chromosomes of each nucleus seem to fuse end to end, but there is no evidence from the material studied that they completely lose their identity at this stage and form a reticulum." In *T. sessile*, as shown in fig. 10, the chromosomes are connected by only a few very small threads.

The cells are the same size at completion of the heterotypic division, and are separated by a complete wall (figs. 10-12). The chalazal cell increases in size more rapidly than the antipodal cell, and usually encroaches on it (figs. 12-20). Sometimes the cells remain almost the same size until after the completion of the homotypic division (figs. 13, 17). When this happens, the four megaspore nuclei are the same size. Often each megaspore nucleus shows six

vacuolate chromosomes with a little anastomosing by very fine threads similar to those formed by the heterotypic division (figs. 14-17). More commonly, however, the megaspore nuclei are reticulate and contain nucleoli (fig. 18). Apparently the megaspores formed in this manner remain in the resting condition for a few days. At no time is there any indication of wall formation between the nuclei formed by the homotypic division. The two micropylar megaspores, although the nuclei often become fully developed, do not function, but soon disintegrate and are absorbed by the rapidly growing chalazal megaspores (figs. 18-20). This is apparent during the homotypic division, which always lags behind that of the chalazal cell (figs. 14-16). In the material observed the homotypic division always begins, but often is never completed (figs. 14-16). According to Miss HEATLEY, in *T. cernuum* disintegration may take place before the beginning of the homotypic division.

The female gametophyte develops from the two chalazal megaspores. At the completion of the homotypic division the nuclei of these megaspores may or may not form netlike reticula and go through a short resting period (figs. 18, 19). The nuclei which do not form resting reticula are similar in appearance to those formed by the heterotypic division (figs. 16, 17), and apparently the two divisions take place immediately to form the 8-nucleate embryo sac, which has enlarged very rapidly (figs. 20, 21). The nuclei take their usual positions (fig. 21) and are now ready for the male nuclei, one of which reaches the egg, and the other the polar nuclei (fig. 22).

The nuclei of the female gametophyte are easily distinguished from the male nuclei by their size, coarse reticulum, and nucleoli. The male nuclei are finely granular, less than one-half the diameter of a polar nucleus, and without nucleoli. Fig. 22 shows a male nucleus in close contact with the two polar nuclei, which are still in the resting condition, although ERNST says that in *T. grandiflorum* the polar nuclei begin to form spiremes even before the male nucleus arrives. He also shows three polar nuclei in *T. grandiflorum*, and says that this occurs occasionally when an antipodal nucleus enlarges and wanders to the center of the embryo sac. Miss NOTHNAGEL states: "In *T. grandiflorum* the three nuclei, which unite to form the primary endosperm nucleus, are all alike in shape, it being

impossible to distinguish the male nucleus by its form or size (fig. 16). . . . All three contain nucleoli; sometimes one, while at other times many." In *T. sessile* the male nuclei differ so much from the other nuclei of the embryo sac that they can easily be distinguished from them, and their identity is certain.

It is evident from this account of the female gametophyte of *T. sessile*, and from those of the two species previously studied, that all have similar origin and development, but that some distinct variations occur. It is likely that food and water supply determine the stage of development of the micropylar megaspores before their disintegration, and whether or not the chalazal megaspores develop immediately after their formation without a resting period. It seems certain that the chromosomes of *Trillium* are vacuolate at all stages of mitosis, since vacuoles were observed throughout the heterotypic division of *T. sessile* in this study, and at metaphase and anaphase in vegetative cells of *T. grandiflorum* by GREGOIRE and WYGAERTS.

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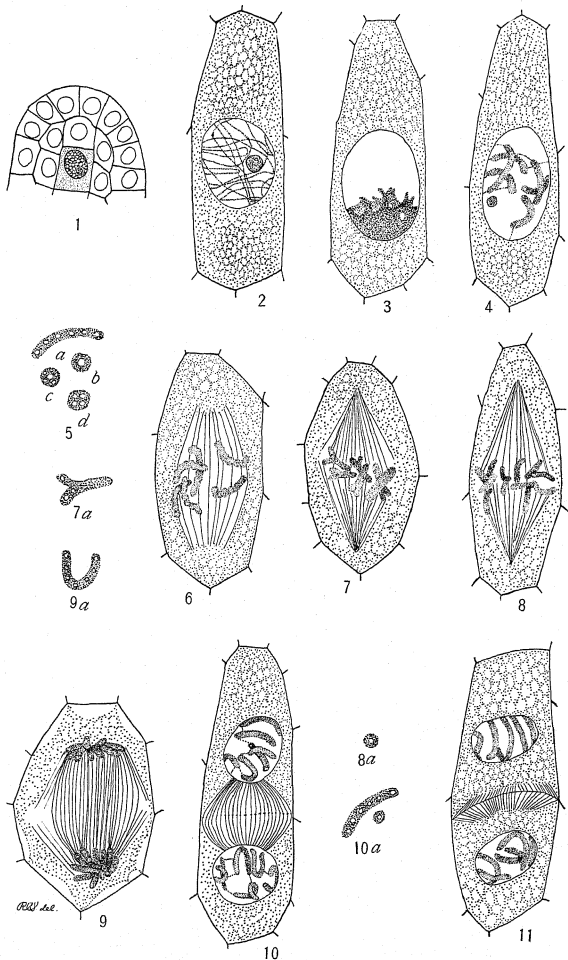
EXPLANATION OF PLATES XVI, XVII

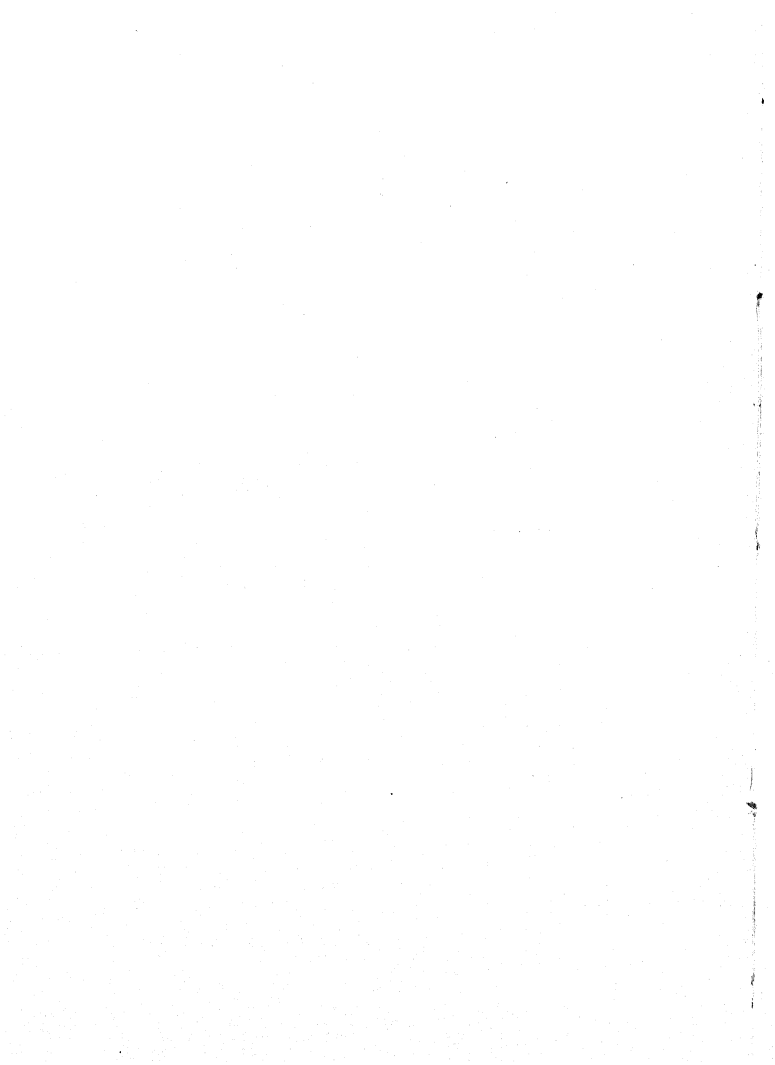
All drawings were made by aid of a camera lucida, except figs. 5, 7a, 8a, 9a, and 10a, which are freehand drawings. Figs. 1, 21, and 22 are magnified 330 times; all other camera drawings 767 times. The micropylar end of all figures is toward the upper edge of the page.

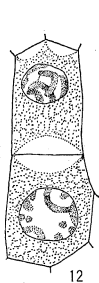
FIG. 1.—Tip of ovule, showing megaspore mother cell.

FIG. 2.—Spireme in megaspore mother cell.

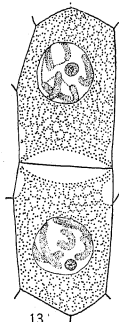
FIG. 3.—Synapsis in megaspore mother cell.



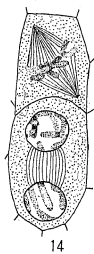




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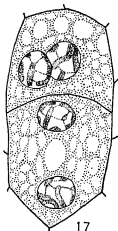
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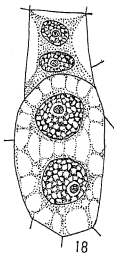
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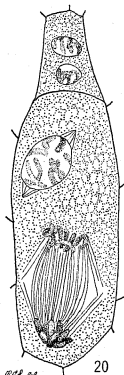
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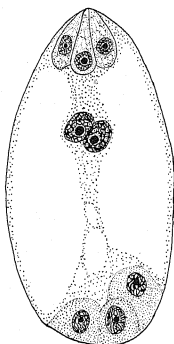
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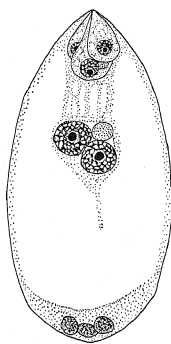
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FIG. 4.—Six chromosomes in nucleus of megaspore mother cell after synapsis.

FIG. 5.—Vacuolate chromosomes from fig. 4.

FIG. 6.—Megaspore mother cell with spindle and chromosomes nearing equatorial region.

FIG. 7.—Metaphase of heterotypic division of megaspore mother cell.

FIG. 7a.—Vacuolate chromosomes from fig. 7.

FIG. 8.—Completion of metaphase of heterotypic division of megaspore mother cell.

FIG. 8a.—Vacuolate chromosome from fig. 8.

FIG. 9.—Anaphase of heterotypic division of megaspore mother cell.

FIG. 9a.—Vacuolate chromosome from fig. 9.

FIG. 10.—Beginning of wall after heterotypic division in megaspore mother cell.

FIG. 10a.—Vacuolate chromosomes from fig. 10.

FIGS. 11-13.—Completion of heterotypic division of megaspore mother cell into two cells.

FIG. 14.—Homotypic divisions; division of micropylar cell lagging.

FIGS. 15, 16.—Homotypic divisions; chalazal complete, micropylar incomplete.

FIG. 17.—Four megaspore nuclei of same size.

FIGS. 18, 19.—Chalazal cell enlarged; micropylar cell disintegrating.

FIG. 20.—Formation of four nuclei by division of two chalazal megaspore nuclei.

FIG. 21.—Mature female gametophyte.

FIG. 22.—Female gametophyte with two male nuclei; antipodal nuclei disintegrating.

BRIEFER ARTICLES

AN UNUSUAL INTRAOVARIAL FRUIT IN CARICA PAPAYA

(WITH ONE FIGURE)

The occurrence of secondary fruits within the ovary of *Carica papaya* L. (papaia) is not uncommon. A description of some of the forms usually found has been published.¹ Very recently a form of much rarer occurrence has been supplied to the writer, through the kindness of Dr. F. G. KRAUSS

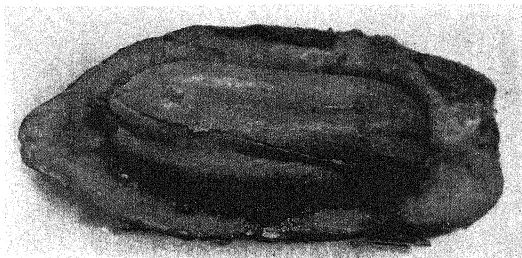


FIG. 1.—Intraovarial fruit of *Carica papaya* as proliferation of stem axis; about $\frac{1}{2}$ natural size.

of the University of Hawaii. This specimen is a model in miniature of a normal fruit. It completely occupied the seed cavity of the fruit in which it was found (fig. 1).

This specimen proved to be a proliferation of the stem axis. Very small fruits occurring as such are occasionally found. An example of this type was described and illustrated in fig. 6 of the former paper. All such forms previously observed have been very small, usually less than an inch long.

The specimen here described measured 8 inches in length and 3.75 inches in diameter. It was pale green, and had a style similar to that of a

¹ Bot. Gaz. 72: 97-101. 1921.

normal fruit. A seed cavity was present but contained no seeds, although minute outgrowths along the placentae indicated the beginning of ovule formation. A similar condition with reference to ovule development is often found in ordinary fruits. In these fruits the failure of the ovules to develop is attributed to lack of pollination. Normally developed seeds were found in the seed cavity of the fruit within which the secondary fruit here described was located.

An examination of the epidermis of the intraovarial fruit showed it to be similar to that of an ordinary papaia, but lacking the waxy coating characteristic of ordinary fruits. Stomata were present but differed from those previously described for intraovarial fruits in being relatively larger, and in having very large round stomatal apertures. The guard cells contained plastids which showed a faint green tinge, to which the color of the fruit was probably due.—H. F. BERGMAN, *University of Hawaii, Honolulu.*

CURRENT LITERATURE

BOOK REVIEWS

Researches on fungi

The third volume of BULLER's work¹ is devoted largely to a consideration of the structure of the hymenium and the method of spore discharge in agarics. The distinctions between the two types of fruit body occurring in these forms, the Aequi-hymeniiferous or non-*Coprinus* type and the Inaequi-hymeniiferous or *Coprinus* type, together with the special characteristics of the *Panaeolus* subtype of the former group, were treated in the second volume, and have been summarized in these pages.² The four remaining subtypes of the Aequi-hymeniiferae are discussed in the volume under consideration, completing the treatment of this series, and the structure of four of the six subtypes recognized in the Inaequi-hymeniiferae is given in detail, leaving two subtypes for treatment in the concluding volume.

Of the non-*Coprinus* forms, the *Psathyrella* subtype is characterized by a short lived basidiocarp, extremely light in structure, and held in shape during the less than twenty-four hours of spore discharge by the turgid, girder-like cells of the open trama. Especially noteworthy is the discovery of the existence in this subtype of tetramorphic basidia, differing in length and with overlapping spores, the longest maturing and shedding their spores first, then collapsing and making way for the three successive spore generations borne on the shorter basidia. The *Bolbitius* subtype has equally ephemeral basidiocarps, but the basidia are monomorphic and not so closely crowded. The deliquescence often noted in these forms takes place only after all the spores are shed, and is wholly different from the progressive autodigestion of the gills occurring in the Coprini. The *Armillaria* subtype is characterized by strongly built, persistent basidiocarps, with monomorphic basidia developing simultaneously over the entire surface of the gills through a period of several days. The fourteen species listed as known to belong to this subtype represent ten genera of white spored and two genera of pink spored agarics. The *Inocybe* subtype has persistent basidiocarps, monomorphic but compactly spaced basidia, and exhibits incipient mottling of the gills. It is intermediate between the *Panaeolus* and *Armillaria* subtypes.

Different species of the same genus may be placed in different subtypes.

¹ BULLER, A. H. R., Researches on fungi. III. The production and liberation of spores in Hymenomycetes and Uredineae. 8vo. pp. xii+611. figs. 227. London: Longmans, Green, & Co. 1924.

² BOT. GAZ. 77:233. 1924.

Thus, *Lepiota procera* seems to belong to the *Armillaria* subtype, while *L. cepae-stipes* is a typical representative of the entirely different *Psathyrella* subtype. *Russula emetica* belongs to the *Inocybe* subtype, while *R. cyanoxantha* and *R. ochroleuca* belong in the *Armillaria* subtype. The structure of the hymenium gives no warrant, in the author's opinion, for classifying either *Bolbitius* or *Psathyrella* with *Coprinus*.

Of the four *Coprinus* subtypes described, the *Comatus* sub-type is characterized by narrow, rigid gills, subparallel sided, with conspicuous flanges at the edges. The basidia are dimorphic and the cystidia are lacking. In the *Atramentarius* subtype the gills are parallel sided, and there are no flanges, but the interlamellar spaces are maintained by numerous cystidia, which act as braces, undergoing autodigestion only just before spore discharge begins in their immediate vicinity. The basidia are dimorphic. In the *Lagopus* subtype the gills are parallel sided, with slightly developed flanges, and are held apart by the numerous cystidia in the younger stages, but separated by the expansion of the pileus before the beginning of spore discharge. The basidia are dimorphic. The *Micaceous* subtype possesses wide, subparallel sided gills with cystidia for the most part clustered at or near their edges, where they act as guards rather than braces in the younger stages, the pileus expanding, as in the *Lagopus* subtype, before the spores are shed. In this subtype the basidia are tetramorphic.

The remaining chapters of Part I are taken up with observations on nocturnal spore discharge in *Pleurotus ostreatus* and *Collybia velutipes*, including notes on the effects of low temperatures; with a complete account of the agarics parasitic upon other agarics, accompanied by a general summary of the parasitism of fungi on fungi; and with a discussion of bioluminescence in fungi.

Part II is concerned with spore discharge in the rusts. It is shown that the mechanism for the discharge of basidiospores (sporidia) in these forms is exactly the same as in agarics. The typically curved shape of the rust basidium (promycelium) is shown to be correlated with the fact that the spores are always borne on the convex outer side, that is, in the position most favorable for spore discharge. The author cites the neglected observations of ZALEWSKI (1883) concerning the violent discharge of acidiospores, which are confirmed by his own observations and by those of DODGE and GROVE. The mechanism of discharge is obviously quite different in this case from that utilized for basidiospore discharge, and tentative suggestions concerning its nature are offered. Uredospores are not violently discharged but accumulate in dusty heaps in the uredosori, from which they are picked up by the wind and distributed exactly as are the spores of puffballs and slime molds.

In this volume and its predecessors, the higher fungi are presented, not as the lowly organized and degenerate forms of the Linnean tradition, but as highly specialized plants displaying minutely adjusted adaptations to their particular way of life. Indispensable to the mycologist, they offer a wealth of material for the consideration of the student of evolution, for the morphologist, and for the

general biologist. In the matter of typography and illustrations the high standard set by the earlier volumes is maintained throughout the third.—G. W. MARTIN.

NOTES FOR STUDENTS

Sex determination.—This subject stubbornly maintains its standing as a live problem in biology, refusing to succumb to any single solution of universal application. The field of investigation is constantly being enlarged, and it is noteworthy that significant data are now being accumulated on sex in plants as well as animals.

The time honored sex chromosome machinery is so easily demonstrated in many bisexual animals, and is so strikingly confirmed by the distinctive method of inheritance of sex-linked characters, that it provides a theory which is almost too satisfactory; for when one is satisfied as to the existence of X- and Y-chromosomes he is in danger of letting the matter rest at that point. This purely qualitative view of sex has been assailed vigorously by RIDDLE and others, who maintain that sex is essentially quantitative, and that the sex chromosomes represent only one of many forces which are operating to determine sex. They urge that purely environmental forces can, through changing the metabolic rate of an organism, convert it into an intersex or even the opposite sex. Recently their position has been strengthened by three cases of complete sex reversal in higher animals, found by CREW³ in the domestic fowl, by CHAMPY⁴ in *Triton*, and by RIDDLE⁵ in the ring-dove. Granting that such transformations are possible, one feels that their effects should be as transitory as are other acquired characters. This impression is supported by the discovery that in frogs, when potential females have been artificially converted into functional males, and are then mated with normal females, the progeny are of the female sex only (CREW⁶). In this mating, every gamete, both sperm and egg, must contain an X-chromosome; evidently the sex transformation which takes place is purely somatic. On the other hand, CREW's transformed hen functioned as father of two chicks, one of each sex. This, however, is what would be expected; in birds the female is heterozygous for sex, so that this mating should produce two females to one male among the viable offspring.

It is only very recently that the sex chromosome machinery has been demon-

³ CREW, F. A. E., Studies in intersexuality. II. Sex reversal in the fowl. Roy. Soc. (London) Proc. Ser. B 95:256-278. 1923.

⁴ CHAMPY, CH., Changement experimental du sexe chez le *Triton alpestris* Laur. Compt. Rend. Acad. Sci. Paris 172:1204-1207. 1921.

⁵ RIDDLE, OSCAR, A case of complete sex-reversal in the adult pigeon. Amer. Nat. 58:167-181. 1924.

⁶ CREW, F. A. E., Sex reversal in frogs and toads. A review of the recorded cases of abnormality of the reproductive system and an account of a breeding experiment. Jour. Genetics 11:141-181. 1921.

strated among plants. ALLEN,⁷ and later SCHACKE,⁸ had provided cytological evidence of X- and Y-chromosomes in *Sphaerocarpus*, but here sex was expressed in the haploid generation, so that the case was hardly parallel with the animal situation. Recently several investigators have found X- and Y-chromosomes (or their equivalents) in dioecious angiosperms. SANTOS⁹ in *Elodea*, KIHARA and ONO¹⁰ in *Rumex acetosa*, BLACKBURN¹¹ in *Melandrium album*, and WINGE¹² in *Humulus lupulus*, *H. japonicus*, and *Melandrium album* (independently of BLACKBURN). WINGE also finds that male plants of *Vallisneria spiralis* have a single sex chromosome (XO), while female plants have two (XX); thus not only the *Lygaeus* type, but also the *Protenor* type of mechanism is shown to exist in the plant kingdom.

Also there was some advance confirmation of these cytological findings by means of sex-linked inheritance. SHULL¹³ and BAUR¹⁴ had found sex-linked inheritance in *Melandrium album*, and CORRENS¹⁵ had demonstrated in striking manner that female-determining pollen tubes were the more rapidly growing in

⁷ ALLEN, C. E., A chromosome difference correlated with sex differences in *Sphaerocarpus*. Science 46:466-467. 1917.

———, The basis of sex inheritance in *Sphaerocarpus*. Proc. Amer. Phil. Soc. 58:289-316. 1919.

⁸ SCHACKE, MARTHA A., A chromosome difference between the sexes in *Sphaerocarpus texanus*. Science 49:218-219. 1919.

⁹ SANTOS, J. K., Differentiation among chromosomes in *Elodea*. Bot. Gaz. 75:42-59. 1923.

———, Determination of sex in *Elodea*. Bot. Gaz. 77:353-376. 1924.

¹⁰ KIHARA, H., and ONO, T., Cytological studies on *Rumex* L. On the relation of chromosome number and sexes in *Rumex acetosa* L. (Eng. résumé p. 86). Bot. Mag. Tokyo 37:147-149. 1923.

¹¹ BLACKBURN, KATHLEEN B., Sex chromosomes in plants. Nature 112:687-688. 1923.

¹² WINGE, O., On sex chromosomes, sex determination, and preponderance of females in some dioecious plants. Compt. Rend. Carlsberg 15:1-26. 1923.

¹³ SHULL, GEO. H., Inheritance of sex in *Lychnis*. Bot. Gaz. 49:110-125. 1910.

———, Reversible sex-mutants in *Lychnis dioica*. Bot. Gaz. 52:329-368. 1911.

———, Sex-limited inheritance in *Lychnis dioica* L. Zeitschr. Indukt. Abstamm. Vererb. 12:265-302. 1914.

¹⁴ BAUR, E., Ein Fall von geschlechtsbegrenzter Vererbung bei *Melandrium album*. Zeitschr. Indukt. Abstamm. Vererb. 8:335-336. 1912.

¹⁵ CORRENS, C., Eine geglückte Verschiebung des Geschlechtsverhältnisses. Botanische Versuche zur Frage nach der Entstehung des Geschlechts. Natur Technik. 2:65-71. 1920.

———, Geschlechtsbestimmung und Zahlenverhältniss der Geschlechter beim Sauerampfer (*Rumex acetosa*). Biol. Zentralbl. 42:467-480. 1922.

M. album and *Rumex acetosa*. ALLEN,¹⁶ also, has recently found a good case of sex-linked inheritance in his *Sphaerocarpus*.

Among those who are opposed to the sex chromosome interpretation among plants, by far the most energetic is SCHAFFNER,¹⁷ who has published numerous papers describing sex-intergrades and complete sex reversal in "normally" dioecious plants.

The possibility of applying the sex chromosome machinery to monoecious plants seems dubious, to say the least. Apparently the only interpretation which there is much hope of applying to all types of cases is that which BRIDGES¹⁸ developed from a genetic and cytological study of inter-sexes in *Drosophila*, and which is enthusiastically accepted by most geneticists. This is really a "sex factor theory" more than a "sex chromosome theory," since sex (potentially quantitative but usually qualitative) is pictured as being influenced by factors upon all chromosomes. Through this theory the essential properties of the previously mysterious X-chromosomes are revealed in terms consistent with the phenomena of Mendelian inheritance, and the possible rôle of the environment in influencing sex is rendered quite plausible. EMERSON¹⁹ is quite sanguine about applying these ideas to the plant kingdom in general, and points out the influence upon sex of several distinct Mendelian factors in maize.—M. C. COULTER.

Cytology of cereal hybrids.—Our most important crop plants, the cereals, have been attracting the attention of cytologists increasingly of late. The fundamental problems of chromosome number and behavior, hybridization, and evolution of species may here be attacked with favorable material, and there is the additional interest that with these cereals something of far reaching economic importance may be achieved.

¹⁶ ALLEN, C. E., Reported at Cincinnati meetings of A.A.A.S. 1923.

¹⁷ SCHAFFNER, J. H., Reversal of the sexual state in certain types of monoecious inflorescences. Ohio Jour. Sci. 21:185-198. 1921.

———, Control of the sexual state in *Arisaema triphyllum* and *A. Dracontium*. Amer. Jour. Bot. 9:72-78. 1922.

———, The sexual nature of vegetative or dichotomous twins of *Arisaema*. Ohio Jour. Sci. 22:149-154. 1922.

———, Sex reversal in the Japanese hop. Bull. Torr. Bot. Club 50:73-79. 1923.

———, Observations on the sexual state of various plants. Ohio Jour. Sci. 23:149-159. 1923.

———, The influence of the relative length of daylight on the reversal of sex in hemp. Ecology 4:323-334. 1923.

———, The time of sex determination in plants. Ohio Jour. Sci. 23:225-240. 1923.

———, Expression of the sexual state in *Sagittaria latifolia*. Bull. Torr. Bot. Club 51:103-112. 1924.

¹⁸ Rev. Bot. Gaz. 72:408-410. 1921; Bot. Gaz. 74:227. 1922.

¹⁹ EMERSON, R. A., A genetic view of sex expression in the flowering plants. Science 59:176-182. 1924.

KIHARA²⁰ deals with an extensive series of hybrids, mostly among the wheats. Chromosome numbers have also been fully investigated, some new determinations being reported. It now appears that wheat falls into three groups: the Einkorn, or Monococcum, having 7 haploid chromosomes, the Emmer having 14, and the Vulgare with 21. The first group is of no economic importance, and hybrids between it and the other groups are sterile. Crosses made between members of the second and the third group give a partially sterile F_1 generation, and an F_2 of various characters and degrees of sterility. The F_1 is found to have 35 chromosomes. In the reduction division, 14 bivalents and 7 univalents are found, the latter splitting in the first division usually, and in the second going indiscriminately to either pole, although belated, and thus often are not all included in a microspore. In the latter case they then disintegrate or form supernumerary pollen grains. Thus pollen is formed whose nuclei possess varying numbers of chromosomes. Sterile combinations are speedily eliminated, and before many generations there are but two numbers represented, 28 and 42, resembling the Emmer and the Vulgare types respectively. Forty-chromosome plants were found which were considered to lack a pair of Vulgare chromosomes. The reduction division in the embryo sac mother cells was found to be identical with that of the pollen mother cells.

Other haploid chromosome numbers found are: oats 7, 14, and 21; barley 7; rye 7 or 8; *Aegilops* 14. Sterile hybrids were obtained between *Triticum vulgare* and rye (*Secale cereale*). The reduction division was somewhat as reported, except that the number of bivalents was variable and the affinity between them weaker. The size of the pollen grain was found to vary directly with the chromosome number.

KIHARA's work as regards wheat is confirmed and in some ways amplified by three other independent publications. WATKINS²¹ is concerned chiefly with the cause of the elimination in subsequent generations, following a cross between wheats of 14 and 21 haploid chromosome numbers, of all chromosome numbers except these two. Two hypotheses are proposed and treated mathematically. In the first it is assumed that the frequencies of the gamete classes are the same in both sexes, and that random mating occurs; in the second it is supposed that in a plant with less than 35 chromosomes, only male gametes with 14 chromosomes function, and in plants with more than 35 chromosomes, only those with 21 function. WATKINS also gives a very careful and detailed account of the reduction division here as observed.

SAX and GAINES²² have made and tested an interesting hypothesis arising

²⁰ KIHARA, HITOSHI, Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B 1:1-200. 1924.

²¹ WATKINS, A. E., Genetic and cytological studies in wheat. I. Jour. Genetics 14:129-171. 1924.

²² SAX, K., and GAINES, E. F., A genetic and cytological study of certain hybrids of wheat species. Jour. Agric. Res. 28:1017-1032. 1924.

out of the situation that in a cross between an Emmer and a Vulgare wheat there are 7 univalent or unpaired Vulgare chromosomes. Characters borne on the latter should not be expected to display Mendelian inheritance, since these chromosome exhibit random distribution with subsequent sterility in varying degree. Characters borne on the 14 primary chromosomes which pair with the Emmer in diakinesis should be expected to show Mendelian inheritance. Thus one would expect normal Mendelian segregation of characters common to the two groups and aberrant segregation of characters which distinguish the groups.

In general the results obtained were in harmony with expectations, although there were indications that in some cases chromosomes carrying distinctive Vulgare characteristics may pair with certain of the Emmer quota. Combinations of Emmer and Vulgare characters were obtained more often in the more fertile combinations, where forms with intermediate chromosome number are not speedily eliminated. No stable combination of favorable characters has yet been secured, however, and the authors are not optimistic of success in this field.

KIHARA'S criticism of BALLY'S report on the hybrid between *Aegilops ovata* and *Triticum vulgare* appears justified in the light of the publication of SAX and SAX²³ regarding crosses made between *A. cylindrica*, a Mediterranean wild grass, and *Triticum vulgare*. The F₂ receives 14 chromosomes from the *Aegilops* and 21 from the wheat parent. At diakinesis there are about 7 bivalents and 21 univalents, due, they think, to the pairing of 7 *Aegilops* chromosomes with 7 wheat chromosomes. The single chromosomes appear to be distributed at random, giving gametes of variable chromosome number, of which only the rarely occurring sets of 7 or multiples thereof can survive apparently. This accounts for the high degree of sterility of the hybrids.

The special characters of the Vulgare group of wheat appear to be due to the 7 additional pairs of chromosomes they possess. These characters are all found in this *Aegilops* species. The remote possibility of a hybrid between *Aegilops* and a wheat of the Emmer series forming a stable race with 21 pairs of chromosomes, and thus establishing the Vulgare group, is favorably regarded. The relative compatibility of chromosomes of different genera is noted.

All these publications are excellently and copiously illustrated, and are substantially in agreement on all points dealt with in common. Since they appeared almost simultaneously on three different continents, they indicate not only the widespread interest in this field, but also the accuracy and the ability of the investigators.—R. O. EARL.

Forests of South Australia.—Recent studies of the vegetation of a portion of South Australia, near Adelaide,²⁴ seem to show clearly how trees of the

²³ SAX, K., and SAX, H. J., Chromosome behavior in a genus cross. *Genetics* 9:454-464. 1924.

²⁴ ADAMSON, R. S., and OSBORN, T. G. B., The ecology of the *Eucalyptus* forests of the Mount Lofty Ranges (Adelaide District), South Australia. *Trans. Roy. Soc. S. Australia* 48:87-144. *pls.* 10-20. 1924.

same genus may appear as dominants in different climatic formation types. The region studied is covered with *Eucalyptus* forests of various kinds, and includes the slopes of a mountain range with a general altitude of 1500 feet, culminating in Mt. Lofy, 2234 feet. The temperature is relatively high and uniform, and 80 per cent of the annual precipitation of 20-40 in. comes during the cool months of April to October inclusive. Various gravels and sands dominate the soils.

In parts of the region where the rainfall exceeds 30 in., *Eucalyptus obliqua* and *E. capitellata* either separately or in mixture form the "stringy bark forest formation." This is a climax forest whose earlier stages are forests of *E. fasciculata* and *E. cleaophora*, or a scrub of *E. cosmophylla* or *Casuarina stricta*. The foliage is of the broad sclerophyll type, the undergrowth is mainly of xerophytic undershrubs, and the degenerate forest and scrub closely resemble the "maquis" of the Mediterranean region. These facts lead the investigators to decide that this forest should be classed as a broad sclerophyll formation, although one of more continental character than those of the Mediterranean region and of California.

In adjacent areas, where the rainfall is less than 30 in., another forest has as dominant trees *E. leucoxylon* and *E. odorata*, mingled with other species of the same genus in various soils. This is also regarded as a climax vegetation for the region under consideration, but on account of its open stand, its herbaceous grasslike undergrowth, and similar conditions it is classified as a "savanna woodland formation." With its various modifications this type is extensively distributed in Australia.

A third forest type, somewhat related to the second in the character of its undergrowth, is termed the "red gum formation," and consists of forests of *E. rostrata*. It is widely spread, occurring along river sides and wherever there is a supply of subterranean water within reach of the roots of the trees. The red gum here appears in a nearly pure stand of large spread trees, whose branches almost touch one another.

In addition to the three climax types, successional stages and edaphic variations are described and brought vividly before the reader in good photographs which are well reproduced. Analyses of the vegetation by Raunkiaer's biological spectra, climatic graphs, and annotated lists of species add to the value of this excellent ecological study.—GEO. D. FULLER.

A basidiomycetous yeast.—Yeasts have occasionally been reported which, when cultured in Petri dishes, produce mirror images of their colonies on the cover of the culture dish, but the reason for the phenomenon seems not to have been investigated heretofore. KLUYVER and VAN NIEL²⁵ have studied three species of yeasts possessing this peculiarity, and find that certain cells of the colonies produce sterigmata, upon which spores are borne, and from which they

²⁵ KLUYVER, A. J., and VAN NIEL, C. B., Spiegelbilder erzeugender Hefenarten und die neue Hefengattung *Sporobolomyces*. Centralbl. für Bakt. 2 Abt. 63:1-20. 1924.

are violently discharged at maturity. The sterigmata are exactly like those of a typical Basidiomycete, although but one is produced by each cell, and the spores are basidiomycetous spores, with a hilum from which a drop of liquid is excreted immediately before discharge, just as in agarics and rusts as described by BULLER. After discharge, these spores proceed to multiply by budding as do ordinary yeast cells. On the basis of this behavior the authors claim that these forms are clearly Basidiomycetes, and they propose for their reception a new basidiomycetous genus, *Sporobolomyces*, without attempting to assign to it any more definite taxonomic position. They quote GUILLIERMOND's generally accepted definition of a yeast as "any unicellular fungus, whatever its biochemical properties, of oval or spherical form, which multiplies by budding." According to this definition, the species of *Sporobolomyces* are clearly yeasts as well as Basidiomycetes.—G. W. MARTIN.

Filmy ferns as indicators of forest conditions.—A recent study of the distribution and habits of growth of the Hymenophyllaceae of New Zealand by HOLLOWAY²⁶ leads to the interesting conclusion that the extent to which ferns generally, and the filmy ferns in particular, adopt the epiphytic habit is a reliable indication of how far the high humidities in the forest interior obtain. It seems to be the lack of a constantly preserved high atmospheric humidity in the southern beech forest, in spite of high rainfall and large number of rainy days, that causes the paucity of epiphytic fern flora in this forest compared with that of the mixed taxad forest. The irregularly shaped tree bases and low spreading horizontal branches in the latter forest are also more favorable to the epiphytic habit.

Many details of the distribution of various species are contained in this report. The species on the outlying islands about New Zealand indicate that none of them possesses any Hymenophyllaceae except such as have resulted from chance dispersal from New Zealand. The family shows few endemics in these regions.—GEO. D. FULLER.

Vegetation of New Guinea.—The recent issue of the Vegetationsbilder²⁷ maintains its high standard of illustrations in depicting portions of the vegetation of the mountains of New Guinea. Among the types illustrated are the conifer forest at altitudes of 3200–4750 m., characterized by species of *Podocarpus*, *Dacrydium*, and *Libocedrus*; openings in the forest with *Trichomanes*, *Gleichenia*, *Cyathea*, and *Alsophila*; scrub associations with *Casuarina* and *Rhododendron*, and a group of myrmecophylous and insectivorous plants including species of *Myrmecodia*, *Hydnophytum*, and *Nepenthes*. A brief descriptive text accompanies the plates.—GEO. D. FULLER.

²⁶ HOLLOWAY, J. E., Studies in the New Zealand Hymenophyllaceae. Trans. New Zealand Inst. 54:577–618. 1922; 55:67–94. 1924.

²⁷ LAM, H. J., Vegetationsbilder aus dem Innern von Neu Guinea. Vegetationsbilder, KARSTEN and SCHENCK 15: Heft 5–7. pls. 25–42. 1924.

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INHERENT FACTORS RELATED TO ABSORPTION OF MINERAL ELEMENTS BY PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 334

ROBERT B. DUSTMAN

(WITH EIGHT FIGURES)

That different species of plants differ widely in their ability to grow on infertile soils has long been recognized by farmers. Rye and buckwheat are well known "poor land" crops, while in many localities scrub pine, sassafras, pennyroyal, broomsedge (*Andropogon virginicus*), and poverty grass (*Danthonia spicata*) are notoriously indicative of impoverished soils. They enjoy this reputation because of their ability to live and thrive where other plants would fail. On the other hand, corn, barley, wheat, and Kentucky bluegrass are generally regarded as "strong land" crops, and into this same general class presumably fall many of the oaks and hickories, the black walnut, and the tall ambrosia (*Ambrosia trifida*).

During recent years much interest has arisen in connection with the manner in which plants absorb mineral elements, the precise nature of the processes involved, and the underlying causes for the differences manifested by different species with respect to the absorption of essential and non-essential elements. From the first investigators have recognized differences in character, size, and extent of

the root systems of plants. It is readily apparent that a plant with a relatively large and actively absorbing area will afford better opportunity for the intake of materials from the soil solution than one with a smaller absorbing area, but as yet we have no very satisfactory method for measuring total areas of root surface exposed, much less a method for determining the actively absorbing portions. In addition to root areas, such factors as concentration of CO_2 in and around the root surfaces, permeability of root cells, acidity of cell sap, and adsorptive and absorptive nature of root colloids need due consideration. In all of these we seem to be still without adequate knowledge of the activities and operation of the root hair. The work herein reported deals chiefly with two phases of the problem, namely, the effect of calcium supply on sap acidity and the intensity of carbon dioxide production by plant roots. Other possible factors receive minor consideration.

Historical

There is an extensive literature bearing upon the general problem of mineral absorption by plants. No attempt has been made to review this literature exhaustively. In the following pages only brief reference is made to the results of certain other workers which relate to root systems, carbon dioxide production, permeability, and the acidity of vegetable saps as influential factors in absorption phenomena.

ROOT SYSTEMS AND ROOT AREAS

Considerable work has been done on the root systems of plants, but there is little accurate knowledge concerning the areas exposed within the soil. In the United States much of the earlier work was done by agricultural experiment station workers. MILLER (22,23) has summarized this, together with certain foreign material, and added to it his own work on corn and the sorghums. More recently, WEAVER and his co-workers have engaged in a systematic investigation of root systems of both agricultural and non-agricultural plants west of the Missouri River. WEAVER, JEAN, and CRIST (49) report that plant roots coming in contact with a fertilized layer of soil retard downward growth, while branching and developing much more abundantly in the fertilized region. WEAVER (48) states that

rye usually has a more branched root system than wheat or oats grown under similar conditions, thus partially explaining why rye will thrive on poorer and more sandy soils than other cereals. In a recent article, WEAVER, KRAMER, and REED (50) report determinations of "total absorbing area exclusive of root hairs" for Kanred wheat, as calculated from numerous measurements of lengths and average diameters. Ordinary laboratory determinations and estimates of the increase in area due to root hair formation, for various seedling plants germinated in moist air, indicate an increase of five to twenty or more times, but the number and development of root hairs normally occurring in soils is less certain, and presumably varies considerably, depending upon conditions. In this connection the early work of SCHWARZ (33), and later work of SNOW (34) and HESSE (13) are suggestive.

All investigations appear to support the statement that there may be wide variations in the comparative extent and development of the root systems of different species of plants.

CARBON DIOXIDE AND PLANT ROOTS

The solvent action of aqueous solutions of carbon dioxide upon soil minerals is too well known to justify discussion in this brief review. The important matter for consideration here is the established fact that plants give off considerable amounts of carbon dioxide from their roots during growth. Since the time of LEIBIG and SACHS it has been known that plant roots will leave their traces upon the surface of marble by etching. There are abundant researches upon the nature of root excretions. The work of CZAPEK (8) gave strong indication that carbonic acid is the only acid of importance normally excreted by the roots of higher plants. From a series of investigations, STOKLASA and ERNEST (37,38) decided that roots of common crop plants give off acids other than carbonic only when normal respiration is disturbed by insufficient oxygen supply. They refute CZAPEK's view that mono-potassium phosphate is excreted in significant quantity from root hairs, and believe that plants are able to absorb relatively insoluble forms of minerals in proportion to the output of CO_2 . KOSSOVITCH (17), working with mustard in solution culture, concluded that CO_2 excretion might in

some cases be important in rendering soil minerals available. ABERSON (1) made electrometric determinations of the acidity of root excretions from young plants of oats, buckwheat, and others, and concluded that, although root excretions contained no noteworthy concentration of H-ion, still the H-ion concentration of a saturated solution of CO_2 , when concentrated in the mucilaginous wall of the root hair, is probably sufficiently effective to dissolve certain soil minerals, particularly the phosphates. HALL (12) had earlier reached a similar conclusion, but from their experiments with crops grown in pots containing different phosphates, and treated with CO_2 gas, PFEIFFER and BLANCK (28) regard any amounts of CO_2 given off by the plant as insufficient to account for the phosphates taken up. NELLER (24) found that growing plants greatly increased the oxidation processes in a soil of average productiveness, as indicated by the CO_2 evolved, and suggests a symbiotic relationship between soil oxidizing organisms and growing green plants. TURPIN (47) determined relative amounts of CO_2 produced by soils growing oats and millet, as compared with uncropped soils. He concluded that the influence of the crop as a producer of CO_2 has been underemphasized, and that the CO_2 output from the plant is largely the result of root respiration, rather than decay of root particles. RUSSELL and APPLEYARD (30) found no significant differences in the CO_2 content of soil air in which different species of plants were growing, but in a later publication (31) they found a considerable increase in the production of CO_2 in the soil during the period of rapid growth of the crop, and another and larger increase at the time of ripening. They consider the latter increase to be inadequately explained as a respiration effect. BIZZELL and LYON (4) determined the period of greatest apparent production of CO_2 by oats to be at the time of bloom, and subsequent to blooming found a marked decrease. This they attribute to a depressing influence of the crop on bacterial action.

The numerous investigations in this field indicate that growing plants give off quantities of CO_2 sufficient in amount to be of importance in the solution of soil minerals in the region of the absorbing roots. The plant may also exert an appreciable effect on oxidation processes in the soil, but the nature of such an effect seems to be somewhat less certain.

PERMEABILITY AND GENERAL ABSORPTIVE PHENOMENA

Many investigations dealing with general absorption phenomena have been made, but comparatively few direct experiments upon the passage of dissolved substances into root cells are available. It is well known that many factors affect the absorption of ions by plants, and also that cell membranes are capable of changes in permeability, depending upon the conditions to which they are subjected. STILES (36) has recently published an exhaustive review of the investigations dealing with permeability in general. The work of MEURER (21), PANTANELLI (26), and others showed that the anions and cations of a salt frequently enter the plant in dissimilar proportion. The work of JOHNSON (15) indicated further that the presence of dead cells may have an appreciable effect upon the relative absorption of the constituent ions. TRUE (40,41) emphasizes the importance of calcium in rendering other elements physiologically available to the plant. HOAGLAND (14) stresses the influence of one ion on the absorption of another, and the importance of conditions of light, temperature, and humidity. Recently COMBER (7) has introduced the idea of a single, intimate, continuous root hair-soil particle colloidal system through which the plant may actually absorb colloidal particles, and whereby the dissolution of soil particles is accomplished by the presence of organic compounds in the root hair so attached. Finally, GERICKE (10) has reached the interesting conclusion that the temporary depletion of certain essential elements in the soil is an important beneficial factor in the production of maximum crop growth.

The evidence from this field of endeavor reveals a complexity of factors and conditions, which at present are not well understood and hence difficult to evaluate, but whose general importance in nutrition and growth presumably is very great.

ACIDITY OF PLANT SAPS

As early as 1907 LEMMERMANN (19) compared the titration acidities of root extracts from a considerable number of different representatives of the grass and legume families. He found characteristic differences, and concluded that the greater acidities generally prevalent in the legumes must be an important factor in their superior ability to secure mineral elements from the soil.

During recent years much interest has been evidenced in the application of H-ion studies to biological problems. In this connection TRUOG and his co-workers have investigated the acidities of various plant saps as determined by the use of the hydrogen electrode. TRUOG and MEACHAM (46) found clover, alfalfa, soy beans, and buckwheat to have less acid saps when grown on limed than on unlimed soils. CLEVINGER (6), however, found the reverse to be true. He states, "with the exception of buckwheat, the tops of the limed plants were usually more acid." He found also that acidity varies somewhat with the stage of development of the plant, and that different portions, as leaves and stems, may yield different results. HAAS (11) showed that there may be an acidity gradient from tops to roots, sweet clover showing acid, neutral, and alkaline saps in the same individual. The range in this case was from P_H 5.82 in the top to P_H 8.00 in the root for a plant in its second year of growth, on soil of P_H 7.68. He showed an increase in acidity with age, and a decrease with a reduction of the normal illumination. BAUER (2) grew various plants under treatment with rock phosphate in sand cultures, and found no definite relation of the amount of plant growth to the extent of the root systems, the phosphorus content, or the acidity of the plant juices. BAUER and HAAS (3) studied the effects of limestone, leaching, phosphate salt, and nitrogen salt on the acidity of the soil medium and plant juices, each treatment showing considerable effect. They state that acidity was closely related to the growth and feeding powers of the plant. KAPPEN and ZAPFE (16) determined the acidity in roots and tops of beans and lupines at blossoming time, when growing on a sandy loam soil under treatment with 1, 5, and 10 per cent additions of calcium carbonate. Sap acidity was not appreciably affected by the additions of limestone, the conclusion being that although plants grown on limed soil probably take up more lime, nevertheless they are able to maintain the same degree of acidity in their juices as on unlimed soil. BRYAN (5) in general secured increased sap acidities with increased acidity of the culture media, but corn tops gave no significant change. Root juices followed culture media much more closely in reaction values than did leaf juices. NEWTON (25), working with barley, peas, and beans in solution cultures, concluded that the

H-ion concentration of the plant sap is not increased by limiting the plant's supply of calcium.

From the data available it would seem that the acidities of expressed saps commonly range from approximately P_H 7.00, for such plants as sweet clover and alfalfa, to about P_H 3.00 for rhubarb and red sorrel. Variations may occur in different portions of the same plant. The evidence as to the effect of external conditions and treatment of culture media on sap acidity is somewhat conflicting.

Materials and methods

EXPERIMENT I: SAP ACIDITY AND CALCIUM SUPPLY.—Since, according to TRUOG, one of the chief functions of calcium in the plant is to neutralize sap acids, it was thought desirable to seek additional light on the matter by studying the effect of calcium supply upon the acidity of the sap of tomato plants. Accordingly, a plan was devised whereby the rates of calcium supply would vary across three reaction values in the culture solution. This is shown in table I, where the numbers in parenthesis represent the numbers of the supply bottles. The plants were grown under greenhouse conditions, with a continuously renewed supply of solution.

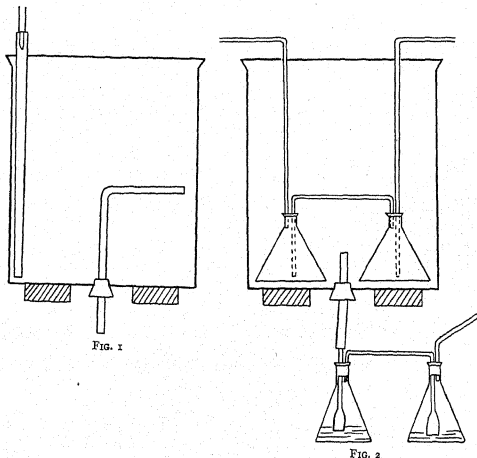
TABLE I
VARIATIONS IN CULTURE SOLUTION

Calcium content	P_H 4	P_H 5	P_H 6
High, 1000 ppm.....	(1)	(4)	(7)
Medium, 100 ppm.....	(2)	(5)	(8)
Low, 10 ppm.....	(3)	(6)	(9)

EXPERIMENT II: CARBON DIOXIDE FROM ROOTS.—In an effort to determine, quantitatively, the production of CO_2 by the roots of representative species of plants, Reid's Yellow Dent corn, Oderbrucker barley, Japanese buckwheat, Rosen rye, Manchu soy beans, and Canada field peas were grown in soil in the greenhouse in jars which were continuously aspirated over a period of six weeks. Weekly determinations of the CO_2 given off by planted and unplanted jars were made. It was desired also to learn something of the size and extent of the root systems developed by the various kinds of

plants, and for this reason dry weight determinations and estimations of relative root areas were made. For purposes of comparison a supplementary trial was arranged, in which the plants also were grown in air-tight containers, in quartz cultures, for a similar period of time.

APPARATUS FOR EXPERIMENT I.—The tomato plants were grown in glazed earthenware jars of 2-gallon capacity, equipped with inlet



FIGS. 1, 2.—FIG. 1, diagrammatic sketch of culture jar used for growth of tomato plants; fig. 2, diagrammatic sketch of soil culture jar with absorption flasks.

and outlet tubes as shown in fig. 1. The culture solution was allowed to enter at the bottom of the jar on one side, and was taken out at a point higher up on the opposite side. Into the upper end of the inlet tube was thrust a piece of smaller tubing drawn to a point. This gave a visible dripping point at which the rate of flow of solution could easily be seen at all times. The rate of flow was

adjusted by means of a screw clamp attached to a piece of extra heavy gum tubing connecting the dripping point with the supply system. The twenty-six jars so equipped were filled with pure quartz sand, and the roots of the growing plants bathed with a continuous flow of the solution described later. This was supplied from nine large glass bottles of 5-gallon capacity. To prevent algal growth the entire system, except for the dripping points and a narrow strip vertically along each bottle, was painted black. The bottles were calibrated in liters in the unpainted strip, thus allowing for additional check upon the flow of the solutions. In addition to the heavy coat of black paint, the supply bottles were doubly wrapped with heavy brown paper. The culture jars were supported 6 inches above the bench upon narrow boards placed a few inches apart, the outlet tubes projecting from the bottoms of the jars down into the opening between the boards. Glass bottles of convenient size were placed beneath the outlets when it was desired to collect and test the solutions coming from the jars.

CULTURE SOLUTION.—In the preparation of a suitable culture solution, it was originally intended to employ potassium hydrogen phthalate as a buffer material, but preliminary experiments showed that it exerts a toxic action on young tomato plants. For this reason it was discarded in favor of sodium carbonate, as described by BRYAN. Calcium was supplied at three different rates. The composition of the unadjusted solution is shown in table II.

The various solutions were made up in nine and eighteen liter quantities, and adjusted to the desired reaction by adding sodium carbonate in suitable quantity, as indicated by the electrometric titration curves. The actual amounts added varied with the reaction values, and to a less extent with the calcium content. Iron was supplied directly to the inlet tubes of the culture jars as ferric phosphate suspension.

SUPPLY OF CULTURE SOLUTION.—The rate of supply of culture solution varied somewhat, due chiefly to temperature changes, but by regulating the screw clamps four to six times daily the variations were not great. At night the flow was much less subject to change. The experiment was begun with about 600 cc. of solution passing through each pot in 24 hours. This was gradually increased

to almost 2 liters when the plants were at their largest. The object was to supply the solution in quantities sufficient to prevent radical changes in the reaction values of that contained within the jars, and that this was accomplished is shown by the data in table III. With some of the pots there was an intermittent siphoning effect, when a considerable portion of the solution would pass out at one time. Others dripped steadily with the inlet tubes.

TABLE II
UNADJUSTED CULTURE SOLUTION

Substance	Grams per liter	Parts of element per million parts of water
H ₃ PO ₄	0.8055	255 of P
KNO ₃	0.8321	321 of K 115 of N
NaNO ₃	0.8440	139 of N 228 of Na
MgSO ₄ 0.7 H ₂ O.....	1.2142	121 of Mg 160 of S
CaCl ₂ High.....	2.7750	1000 of Ca 1770 of Cl
Medium.....	0.2775	100 of Ca 177 of Cl
Low.....	0.0277	10 of Ca 17 of Cl

METHOD OF GROWING PLANTS.—On January 16 seeds of the Bonny Best variety of tomato were sown in soil and the plants repotted from time to time during the next four months. During this period the most vigorous plants only were saved, one being chosen eventually as the stock plant from which the cuttings were made. Preliminary trial showed that cuttings could satisfactorily be rooted in tap water and subsequently transferred to sand or soil as desired. On July 14 the cuttings were set in the culture jars. At this time also thirteen of the twenty-six jars were planted to seeds of the STONE variety, and these later thinned to six plants per jar. Supply bottles nos. 2, 4, 6, and 8 supplied four culture jars each,

while nos. 1, 3, 5, 7, and 9 supplied only two jars each. In each case the number of jars growing cuttings and seedlings was the same, or a total of thirteen for each.

PREPARATION OF MATERIALS AND DETERMINATION OF REACTION VALUES.—In all determinations of reaction values the standard Leeds and Northrup Type K potentiometer was used, equipped with a bubbling hydrogen electrode of the Hildebrand type, and a saturated calomel electrode with liquid junctions across saturated KCl solution. Plant saps were secured by the usual method. After harvest the plants were immediately frozen solid, macerated in a meat chopper (or mortar where the quantity of material was small),

TABLE III
P_H RANGE OF CULTURE SOLUTION

SUPPLY BOTTLE	CALCIUM CONTENT (PPM)	AS MADE UP	ISSUING FROM CULTURE JARS	
			Cuttings	Seedlings
1	1000	4.01-4.05	4.80-4.87	4.93-5.05
2	100	4.07-4.10	5.34-5.40	5.25-5.37
3	10	4.27-4.28	5.15-5.30	5.00-5.33
4	1000	4.90-4.96	5.05-5.16	5.08-5.20
5	100	5.35-5.40	5.44-5.55	5.51-5.55
6	10	5.30-5.40	5.47-5.79	5.25-5.51
7	1000	5.35-5.45	5.19-5.30	5.21-5.35
8	100	5.96-6.05	5.77-6.00	6.03-6.18
9	10	6.03-6.12	5.96-6.15	5.97-6.22

folded in muslin cloths, and pressed in a hand press. The expressed juices were then centrifuged before the determination of reaction values. In a few cases where, due to the poor growth of the plants, the quantity of sap secured was quite small, it was diluted with an equal volume of redistilled water.

APPARATUS FOR EXPERIMENT II.—In this experiment also, the soil cultures were arranged in glazed earthenware jars of 2-gallon capacity with perforated bottoms, as already described. Each jar contained two auto-irrigator cones of porous clay, as described by LIVINGSTON (20) (fig. 2). These were connected in series within the jars and eight jars joined together, all supplied with distilled water from a single source, and all drawing their moisture against a single U-tube of mercury. At the lowest point of the bend the U-tube was

provided with a small outlet tube closed by a pinchcock. Through this, mercury was withdrawn from time to time as the plants increased in size and rate of transpiration. Except for a defective rubber connection between the cones in one of the unplanted jars, which was easily and quickly repaired, the entire system worked

admirably. Small quantities of air accumulated in the tubes frequently, and were readily removed by drawing them forward through the system. Continuous aspiration over the six weeks' period was accomplished by the use of the large bottles described in the preceding experiment, and subsequently converted into aspirator bottles. The rate of flow was regulated by means of small-bore glass stopcocks attached at the lower end of the outlet tubes.

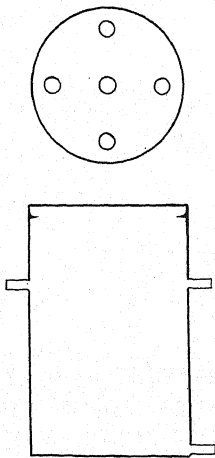


FIG. 3.—Galvanized iron tank used as air-tight container in experiment II.

In the supplementary trial the same kinds of plants were grown in galvanized iron containers of 2.5 gallons capacity, designed especially for the experiment as shown in fig. 3. The tanks were coated inside with paraffin, filled with pure quartz sand, the high calcium culture solution already described added, and the tops sealed off with a heavy layer of paraffin. In this holes were cut through which the plants were grown, and when they had attained sufficient size the openings around the stems were filled with a suitable mixture of plastic clay and vaseline. It was found that by leaving the holes slightly sunken and keeping the depressions filled with water, no particular difficulty was experienced in maintaining the containers air-tight. Without the covering of water the clay tended to dry out and crack with the expansion of the plant stems. The small amounts of water so held likewise gave excellent indication of the tightness

of the seals about the stems. Carbon dioxide-free air was drawn across the roots of the growing plants. Fresh culture solution and distilled water were added frequently. Aspiration and CO_2 determinations were similar to those described for the plants grown in soil.

ASPIRATION OF CULTURE JARS.—The aspirator bottles were accurately calibrated in liters, by which means the flow of air through the culture jars was kept practically constant. Nevertheless, constant care and attention were required, and even so there would sometimes be differences of as much as a liter in the amount of water dripping from the bottles during the night. Dissolved air in the tap water used was the greatest source of trouble. It was necessary to regulate the flow as frequently as five or six times daily in order to keep the bottles emptying together. When differences occurred they were always adjusted within a few hours, so that the total aspiration was the same for all jars within any given twelve-hour period. The rate of aspiration was increased slightly from week to week as the plants grew.

ABSORPTION AND DETERMINATION OF CARBON DIOXIDE.—Absorption of CO_2 was accomplished by means of two flasks of $\text{N}/10$ barium hydroxide. The first flask was given the bulk of the hydrate solution, and the second flask served as a guard against saturation of the solution over night and consequent loss of CO_2 . A few drops of phenolphthalein indicated the alkalinity of the solutions. Sufficient tenth normal barium hydrate was placed in the flasks to last throughout the week. At the end of the 7-day period the excess hydrate was titrated, using oxalic acid of approximately tenth normal strength, freshly standardized, and phenolphthalein indicator. The oxalic acid was standardized by titrating against the tenth normal barium hydroxide, and the latter in turn checked by titration with tenth normal HCl . At first straight barium hydroxide was used in the absorption flasks. The solubility of BaCO_3 in this solution was at times appreciable, however, necessitating a retitration and slight correction for certain of the flasks after standing. Consequently all later work was carried out using barium hydrate solution containing 1 gm. of barium chloride per liter. This corrected the difficulty and gave satisfactory results.

PREPARATION OF SOIL AND JARS.—The soil used was a very fine sand moderately high in well decomposed organic matter, which lent itself well to thorough mixing and subsequently to the removal of the plant roots. It was fertilized with 2000 lbs. of a 5-12-5 fertilizer per 2,000,000 lb. of soil, the fertilizer being added in the form of NaNO_3 , KNO_3 , and mono-calcium phosphate. Eight jars received 20 lb. of soil each, a ninth jar being filled with pure dry sand, through which air was aspirated similarly to the soil pots, in order to determine the CO_2 content of the air passing through the soil cultures. After carefully filling and packing the soil uniformly about the irrigator cones, the jars were joined together and the water system drawn full. They were allowed to stand for the next eight days, during which time moisture had appeared on the surfaces of all. Aspiration on the unplanted jars was then begun and continued for an 8-day period, in order to determine the behavior of the individual jars with regard to CO_2 production before being planted. The results are shown in table VII. As already indicated, pot no. 2 developed a leak in the rubber connection between the cones, and it was necessary to remove about one-fifth of the soil from the pot, in the shape of a central core, in order to repair the connection. The soil was replaced and packed as carefully as possible.

GROWTH OF PLANTS.—At the close of the 8-day run the pots showing the greatest and least production of CO_2 , nos. 9 and 2 respectively, were chosen as checks and remained continuously unplanted throughout the experiment. The remaining six were planted on February 24. By March 2 all plants were well up and were thinned to ten per jar, except corn, of which five plants were allowed to grow. A layer of sand 1 inch thick was placed on the pots to reduce evaporation. Growth was very rapid for all plants during the early period. By March 28 the buckwheat was in full bloom and the soy beans beginning to bloom also. The barley showed a tendency toward prostrate growth, and the rye somewhat less with more tendency to tiller. The temperatures prevailing in the greenhouse seemed somewhat to retard the normal development and bloom of the peas. Aspiration was discontinued April 8, and the following day the plants were harvested.

METHOD OF SECURING AND HANDLING ROOTS.—After removing the tops of the plants, the roots were carefully washed out over a fine screen, using a low pressure stream of water. They were collected, freed from clinging particles of organic matter and sand by washing through several changes of distilled water until they gave solutions free of turbidity, and used for estimations of relative areas as follows. A rough approximation was made of the relative areas involved, and a corresponding quantity of a solution of methylene blue, of 50 mg. per liter strength, measured out. The roots were pressed lightly between pieces of filter paper to remove excess moisture, and dropped into the dye solution where they were kept under frequent agitation. At intervals of 10, 20, and 40 minutes, 5 cc. samples were withdrawn, diluted to a convenient range, and compared with standard color tubes made up from the original stock solution employed for the roots. From the decrease in concentration of the dissolved dye the quantity taken up was calculated, and this used as an indication of the relative areas involved, as explained later. The quantities used and the results obtained are shown in table IX. Later the roots were dried and the dry weights determined by deducting for the amount of dye absorbed.

Results

A. EFFECT OF CALCIUM SUPPLY UPON ACIDITY OF EXPRESSED SAP

The culture solutions for the nine supply bottles were made up with varying calcium content and additions of sodium carbonate. After standing for 24 hours or longer, each newly made supply was tested for reaction value. Also samples of the used solutions were caught once each week as they issued from the culture jars and their reaction values determined (for range, see table III).

The shift in reaction value of the culture solution from that contained in the supply bottle to that leaving the culture jars has usually been toward the alkaline side of the scale. A preliminary run of one week before the jars were planted also gave changes in reaction value, probably due to the activities of organisms present. The discrepancy in the P_H value of supply bottle no. 7 with the calculated value is largely due to the precipitation of calcium phosphate which occurred in the high calcium solution at P_H 6.

Table IV shows, with one exception, a slight increase in the acidity of the expressed saps with decreasing calcium supply. This is in accord with the theory earlier suggested by TRUOG (43,44), and later developed by PARKER and TRUOG (27), but the significance of

TABLE IV
REACTION VALUES OF EXPRESSED SAPS (GROWTH PERIOD 31 DAYS)

SUPPLY BOTTLE	CALCIUM CONTENT (PPM)	P _H VALUE OF EXPRESSED SAP		AVERAGE GREEN WEIGHT PER PLANT OF SEEDLINGS (GM.)
		Cuttings	Seedlings	
1.....	1000	5.77	5.60	6.1
2.....	100	5.55	5.57	1.5
3.....	10	5.35	5.50	0.4
4.....	1000	5.36	5.57	4.0
5.....	100	5.42	5.50	0.4
6.....	10	5.42	0.3
7.....	1000	5.41	5.61	3.0
8.....	100	5.38	5.56	1.3
9.....	10	5.51	0.3
Grown in rich soil.....		5.40	6.00	31.7

PLANTS GROWN IN RICH SOIL

STOCK PLANT DETERMINATIONS

P_H VALUE OF SAP

Seven months old woody stem without leaves.....	5.41
Somewhat younger stem without leaves.....	5.36
Medium stem with its leaves (duplicates).....	{ 5.02 5.00
Tip shoots, tender stems and leaves (duplicates).....	{ 4.95 4.95

SEEDLING PLANT DETERMINATIONS

One month old, large, thrifty, turgid (duplicates).....	{ 5.74 5.51
Same but wilted 24 hours (duplicates).....	{ 5.53 5.50

the differences appears to be of doubtful value. It will be noted that the different portions of the stock plant varied as much as 0.4 of a P_H unit in the value of its juices, and individual seedlings grown side by side in soil for a period of one month showed corresponding variations of 0.2 of a P_H unit. Most of the differences accompanying the changes in calcium supply as shown in table IV are of a

lesser order of magnitude. Reference to the average green weights of the seedling plants shows that their growth was enormously affected by the differences in calcium supply, and at any given reaction of the culture solution the acidity of the sap decreases with increasing growth. Both the differences in calcium supply, however, and in the corresponding growths secured seem out of all proportion

TABLE V
REACTION VALUES OF SAPS FROM PLANTS GROWN UNDER
REVERSED CONDITIONS OF CALCIUM SUPPLY
(SECOND GROWTH PERIOD 15 DAYS)

Former P_H	Direction of change	P_H value of additional growth
Cuttings		
5.35	Low to high	5.78
5.38	Medium to high	6.21
5.77	High to low	6.02
5.36	High to low	5.70
5.41	High to low	5.73
Six weeks old cutting grown in soil		5.57
Seedlings		
5.50	Low to high	5.99
5.42	Low to high	5.99
5.56	Medium to high	5.90
5.60	High to low	5.53
5.57	High to low	5.68
5.61	High to low	5.80
Six weeks old seedling grown in soil		5.55

to the differences in sap acidity, although the seedling grown in soil shows both the greatest growth and the least acid sap.

When the plants grown for a time under conditions of high calcium supply were changed to a low calcium solution, the resulting change in sap acidity was not toward a more acid sap, even though their growth was promptly checked by the decreased supply of calcium. Instead there seems to have been a steady march toward less acid saps, regardless of the direction of change of calcium supply. In this connection it is to be noted that the older portions of the stock plant show the less acid juices. In the light of these later

trials, it would seem that the differences first mentioned are of questionable significance, and that there is no simple direct relation between calcium supply and the acidity of the expressed sap.

B. PRODUCTION OF CARBON DIOXIDE BY GROWING PLANT

The first carbon dioxide measurements made were discontinued after three weeks duration, because of lack of uniformity of conditions within the various pots and consequent inconsistency of results. In this first trial no auto-irrigators were used, and in spite of frequent weighings and corresponding additions of water, considerable

TABLE VI
ASPIRATION RECORD OF SOIL JARS

DATE	LENGTH OF PERIOD IN DAYS	AIR ASPIRATED THROUGH JARS	
		Total (liters)	Daily average (liters)
February			
13-20.....	8	30	3.7
21-25.....	5	18	3.6
26-March 3.....	7	28	4.0
March			
4-10.....	7	30	4.3
11-17.....	7	31	4.4
18-24.....	7	32	4.6
25-31.....	7	32	4.6
April			
1-7.....	7	33	4.7

fluctuation in moisture was experienced, which seemed to be reflected in the quantities of CO_2 collected from the pots. Adding the water in bulk lots to the planted jars gave rise to considerable variation in distribution, while the checks went forward practically without change from day to day and week to week. A serious difficulty was encountered in the tendency of the soil to shrink away from the sides of the jar following a period of heavy transpiration. This introduced the possibility of serious alteration in the method of aspiration. It was also apparent that even with the utmost care the frequent handling of the jars necessitated by the constant weighings was not best for the plants. However, the run furnished valuable experience upon which the later technique was built. As

already explained, jars equipped with auto-irrigators were finally employed. The rates of aspiration over the eight weeks' period and the CO_2 collected from the various pots are shown in tables VI and VII A, B.

The rate of approximately 4.5 liters daily was arbitrarily chosen, after a number of trials with different rates on uniform pots of bluegrass. It seemed sufficiently rapid to give a constant drift of air downward through the jars, and also sufficiently slow to permit good uniformity of flow from the aspirator bottles.

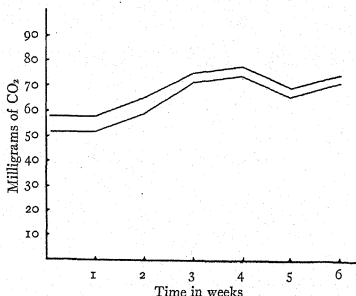


FIG. 4.—Production of CO_2 by soil check pots

In view of the divergence in CO_2 production shown during the first 8-day period between pots nos. 2 and 9, it is of interest to follow their behavior throughout the period of growth of the plants. This is shown by fig. 4. It will be seen that the initial difference is constantly lessened during the progress of the experiment, which seems to justify the method of calculation used. Thus the quantity of CO_2 given off or induced by the growing crop has been calculated by deducting the value of the air check and the average value of the two soil checks from the total production of the pot in question.

C. ROOT SYSTEMS AND ROOT AREAS

In table VIII the green and dry weights of tops and roots are shown, while table IX gives the results of the attempted measure-

ments of relative root areas on the basis of the 10-minute absorption period. The figures for the longer absorption periods lead to similar results.

TABLE VII A

PRODUCTION OF CARBON DIOXIDE BY PLANTS GROWN IN SOIL
(FIRST 8 DAYS, ALL POTS UNPLANTED)

Pot	CONTENT	MILLIGRAMS OF CO ₂ COLLECTED		
		Total	From soil	Daily average
1.....	Dry sand	21.8
2.....	Soil	92.5	70.7	8.84
3.....	Soil	100.5	78.7	9.84
4.....	Soil	97.4	75.6	9.45
5.....	Soil	101.0	79.2	9.90
6.....	Soil	97.2	75.4	9.42
7.....	Soil	94.8	73.0	9.13
8.....	Soil	97.2	75.4	9.42
9.....	Soil	102.6	80.8	10.10

TABLE VII B

FIRST 6 WEEKS OF GROWTH PERIOD

Pot	Kind	MILLIGRAMS OF CARBON DIOXIDE						
		First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Total
		Total from unplanted jars						
1.....	Air check	21.1	24.4	22.4	23.8	24.9	26.4	143.0
2.....	Soil check	73.0	83.3	94.6	98.0	90.3	97.9	537.1
9.....	Soil check	79.0	89.6	98.0	101.6	94.2	100.8	563.2
		Increases due to plants						
3.....	Corn	-9.1	6.3	20.9	78.3	104.9	179.0	380.3
4.....	Barley	4.1	7.6	11.5	24.3	25.9	39.1	112.5
5.....	Buckwheat	7.4	10.1	14.1	20.8	38.1	58.3	157.8
6.....	Rye	3.8	9.6	13.0	23.6	28.1	54.9	133.0
7.....	Soy bean	15.0	21.0	29.9	48.2	62.3	75.1	251.5
8.....	Field pea	14.4	21.0	17.9	27.2	25.2	37.7	143.4

Table X shows what may be termed the coefficients of carbon dioxide production for the various plants secured in two ways: (1) by dividing the total CO₂ production by the figure representing the relative quantity of dry matter of the roots; and (2) by dividing

the total production of CO_2 by the quantity representing the relative root area as determined. The order of sequence of the crops is the same in either case, although the corresponding ratios differ some-

TABLE VIII
GREEN AND DRY WEIGHTS OF CULTURE PLANTS

PLANT	TOPS		DRY ROOTS (GM.)	RELATIVE DRY WEIGHTS (ROOTS)
	Green (gm.)	Dry (gm.)		
Corn.....	147.1	19.587	5.7082	13.30
Soy bean.....	69.6	16.500	1.7196	4.00
Barley.....	43.0	5.231	1.3893	3.24
Rye.....	30.8	4.323	0.5897	1.37
Buckwheat.....	65.8	8.857	0.4617	1.07
Field pea.....	31.7	4.090	0.4291	1.00

TABLE IX
ESTIMATION OF RELATIVE ROOT AREAS

Plants	Dye used (cc.)	Dye absorbed (mg.)	Relative absorption
Corn.....	1600	53.8	6.8
Soy bean.....	800	30.9	3.9
Barley.....	440	15.1	1.9
Rye.....	380	10.3	1.3
Buckwheat.....	200	8.3	1.05
Field pea.....	200	7.9	1.00

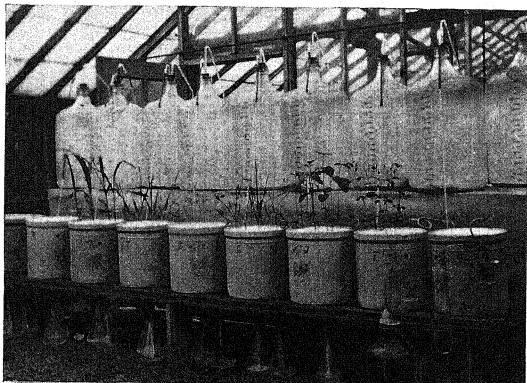
TABLE X
COEFFICIENTS OF CARBON DIOXIDE PRODUCTION

Plant	Basis of root dry matter	Basis of estimated areas
Corn.....	28.6	55.9
Barley.....	34.7	59.2
Soy bean.....	62.9	64.5
Rye.....	97.1	102.3
Field pea.....	143.4	143.4
Buckwheat.....	147.5	150.3

what among themselves. This is particularly true for corn and barley, which plants also possessed considerably larger and heavier main roots than the others.

It is not assumed by the writer that the method of dye absorption gives a true measure of the relative total areas involved. Indeed,

careful observations seem to indicate that it may more nearly approximate active absorbing areas or those areas exposed by the finer roots of the plant. The larger the diameter of the roots, the less the



5



6

FIGS. 5, 6.—Fig. 5, soil culture jars showing method of arrangement and plants two weeks after planting: from left to right, corn, barley, buckwheat, rye, soy beans, field peas; fig. 6 same as fig. 5, four weeks after planting.

amount of dye per unit area that seemed to be concentrated on their surfaces. The smaller roots were darker following treatment with the dye. Previous trials with varying amounts of corn and soy bean roots indicated that for a given kind of root the amount of dye taken up, under the conditions of the experiment, was proportional to the surfaces exposed. There may be specific effects depending upon the nature of the roots, but at present this method of attack

TABLE XI
CARBON DIOXIDE FROM PLANTS GROWN IN QUARTZ CULTURES
(PLANTS 12 DAYS OLD WHEN ASPIRATION BEGAN)

TANK	KIND	MILLIGRAMS OF CARBON DIOXIDE				
		First week	Second week	Third week	Fourth week	Total
1.....	Unplanted	821	749	689	844	3103
Increases due to plants						
2.....	Corn	884	927	902	1113	3826
3.....	Buckwheat	271	365	416	425	1477
4.....	Soy bean	532	630	770	829	2761
5.....	Field pea	741	789	901	617	3048
6.....	Barley	468	637	733	804	2642
7.....	Rye	354	525	765	586	2230

appears promising, and is far less laborious than that of compilations from measurements of lengths and average diameters. In connection with possible specific effects, it was noted that in both experiments the corn roots were of a lighter shade than those of the other plants after exposure to the dye.

D. PLANTS GROWN IN QUARTZ CULTURES

Tables XI-XIV give the results secured from the plants grown in quartz cultures. In this case there was not the tendency toward prostrate growth previously described, but both rye and barley tillered freely. The growth of the rye and buckwheat seemed less vigorous than that of the other plants. There was always free culture solution standing in the bottoms of the metal containers.

The results obtained with the quartz cultures are in general agreement with those secured from the soil cultures, although less

consistent than the latter. A quite unexpected result was the very much greater production of CO_2 by the quartz cultures. A study of the tables shows that both the plants and the culture medium

TABLE XII
GREEN AND DRY WEIGHTS OF QUARTZ CULTURE PLANTS

PLANT	TOPS		DRY ROOTS (gm.)	RELATIVE DRY WEIGHTS (ROOTS)
	Green (gm.)	Dry (gm.)		
Corn.....	140.2	14.872	3.249	12.94
Buckwheat.....	16.1	2.198	0.251	1.00
Soy bean.....	43.0	8.503	2.171	8.65
Field pea.....	24.2	2.988	0.892	3.55
Barley.....	43.7	4.860	1.392	5.55
Rye.....	23.7	3.590	1.025	4.08

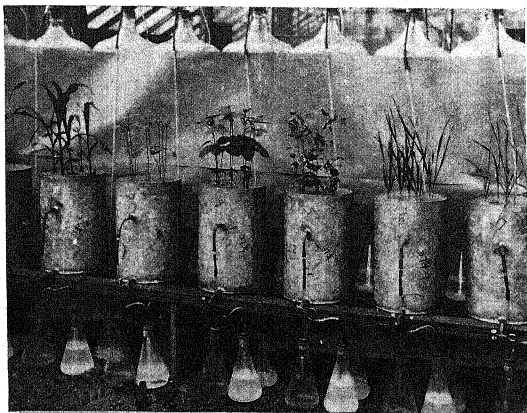
TABLE XIII
ESTIMATION OF RELATIVE ROOT AREAS

Plants	Dye used (cc.)	Dye absorbed (mg.)	Relative absorption
Corn.....	1600	33.3	5.8
Soy bean.....	1000	23.2	4.0
Barley.....	720	14.7	2.5
Rye.....	640	13.2	2.3
Field pea.....	400	8.8	1.5
Buckwheat.....	200	5.8	1.0

TABLE XIV
COEFFICIENTS OF CARBON DIOXIDE PRODUCTION

Plant	Basis of root dry matter	Basis of estimated areas
Corn.....	295	660
Soy bean.....	319	690
Barley.....	476	1060
Rye.....	546	970
Field pea.....	858	2030
Buckwheat.....	1477	1477

(solution) contributed to the increase. The reason for this behavior is not entirely clear. The number of plants per culture was the same in either case, and the difference in size of containers was slight. It is not possible to account for the differences on the basis of loss by diffusion from the soil jars.



7



8

FIGS. 7, 8.—Fig. 7, air-tight containers with plants sixteen days after planting: from left to right, corn, buckwheat, soy beans, field peas, barley, rye; fig. 8 same as fig. 7, four weeks after planting.

E. PERMEABILITY AND SPECIFIC ABSORPTIVE NATURE
OF PLANT ROOTS

The rôles played by permeability and the specific adsorptive nature of root colloids in the absorption of minerals by plants are still unsolved problems. The field is a very complicated one, and difficult to submit satisfactorily to experimental test. In trials with the absorption of copper salts from solution by different kinds of roots, it was noted that barley seemed always to take up more of this element in a given length of time than equal masses of certain other kinds of roots. Following this lead, an attempt was made to measure the relative quantities of calcium and potassium absorbed during very brief intervals of five to ten minutes from equivalent concentrations of their bicarbonates. Most of the plants tried seemed to remove both elements in equivalent amounts, but barley and bluegrass took up approximately three times as much calcium as potassium, whereas rye took up twice as much potassium as calcium. The extent to which ion exchange may have entered in was not determined, and the experiments were not repeated. It is very difficult to know when, if ever, plant roots are free from adsorbed materials on their surfaces, and the significance of the results is therefore obscure. Nevertheless the possibility of importance of these factors cannot be denied, and it is hoped that future work will throw more light on their significance.

Discussion

An effort has been made to consider various factors that may be involved in the absorption of mineral elements by plants, and evidence bearing upon certain of these factors is presented. In connection with the relation of calcium supply to the acidity of the plant sap, if one of the chief functions of calcium is to neutralize by-product acids in the plant, it seems reasonable to suppose that limiting the plant's supply of this element would result in appreciable increases in the acidity of the expressed sap. Just how great such increases would be is somewhat problematical. Probably they would not be pronounced, yet it would seem that they should be capable of measurement and of greater degree than the moderate variations commonly observed in normal plants. It is apparent that the

results herein reported do not bear out, although they do not necessarily disprove, the theory advanced by TRUOG and his co-workers. It is true that analyses were not made to show that the plants actually took up more calcium from the high calcium solutions, but this was done by NEWTON with results in general agreement with those given here. In this work the tomato was used as a culture plant because of the large quantity of juices normally present in its tissues, and because of the possibility of securing a large number of cuttings from a single plant, thus minimizing the differences so frequently encountered due to individual variation.

With respect to the relation of calcium to organic acids in plants, it is interesting to examine some of the ideas prevalent in the literature. As already stated, PARKER and TRUOG suppose one of the chief functions of calcium to be the neutralization and removal of excessive and harmful acids formed as by-products during normal growth processes. On the other hand, THATCHER (39) believes the deposition of calcium oxalate crystals to be a device for the avoidance of excessive calcium in the plant juices. This latter view is held also by GERHARDT (9) and by STAHL (35), these investigators associating calcium oxalate formation with guttation, and the presence of mycorrhiza as a means of removal and prevention of excessive salts, especially those of calcium. Whatever their true rôles may be, it appears that present knowledge is insufficient to support a statement of direct functional relation between calcium and organic acids in plant juices.

The results dealing with carbon dioxide measurements are in general accord with those reported by other writers. LAU (18) found a greater production of CO_2 in soils growing potatoes and lupines than in those growing cereal crops, while STOKLASA and ERNEST (37) found buckwheat, oats, and rye to produce more CO_2 per unit of dry weight of roots than wheat or barley. TRUOG (45) apparently considers the differences in CO_2 production by plant roots as insufficient to account for the differences in growth response commonly observed, although the solvent action of CO_2 at root surfaces is by no means ignored. He emphasises (42) the fact that the conditions obtaining in root cell and root hair surfaces have never been duplicated in laboratory experiments with CO_2 . Produc-

tion of CO_2 is apparently an expression of metabolic activity, and different species of plants differ considerably in this respect. This is also true when relative root masses and recoverable root areas are considered. Buckwheat shows a very meager root system in proportion to its total dry weight, yet it gives off relatively large quantities of CO_2 , which must result in uniformly higher concentrations of carbonic acid about its absorbing roots than is the condition with many other plants. The work of SCHLOESING (32) suggests the possibility of an increasing rate of solubility of certain minerals with increasing concentrations of CO_2 . Continued removal of the dissolved substances by the plant would result in a still greater effect of the CO_2 excreted. It is at least interesting to observe that the order of sequence of the crops with respect to the intensity of CO_2 production, as shown in table X, is very similar to that shown by actual farm experience in their ability to grow on infertile soils. It would doubtless be erroneous to attribute differences in the native capacity of plants to absorb mineral elements from the soil to differences in carbon dioxide production alone. More probably there are many factors involved of which carbon dioxide is an important one.

It is unfortunate that we have no accurate knowledge of the production and distribution of root hairs for the various kinds of plants growing under normal conditions in soils. Consequently we are forced to base opinions upon the recoverable portions of the root systems only, which may not necessarily be proportional to total absorbing surfaces. In his report on corn and the sorghums, MILLER states that, judging from the number of secondary roots found, certain sorghums have a root system twice as efficient as corn in the absorption of water from the soil. Comparative extent of root systems is probably important in determining differences in absorption by plants otherwise similar in metabolism, composition, and growth, but that it cannot be the only factor bearing upon this point, or even a dominant one under all conditions, is readily seen by a comparison of the root systems of two such plants as barley and buckwheat. The production and operation of root hairs, together with permeability and colloidal absorptive effects, are in need of much further investigation.

Summary

Studies were made of the effect of varying calcium supply upon the acidity of the expressed sap of tomato plants, and of the production of carbon dioxide by the root systems of common crop plants. The outcome of these have been as follows:

1. Limiting the plant's supply of calcium, even to the point where cessation of growth and death resulted, did not increase the H-ion concentration of the expressed sap in significant degree.
2. When plants supplied with a high calcium solution were transferred to one of low calcium content the expressed saps did not show a corresponding increase in acidity, although the nature of the change was sufficiently radical to retard their growth.
3. Irrespective of the direction of change of calcium supply, the saps of the plants so treated became somewhat less acid.
4. Moderate variations in reaction values were found to occur in the expressed saps from different individuals and from different portions of the same plant.
5. The stock plant from which the cuttings were taken showed a decreasing acidity gradient with increasing age of the tissues.
6. Considerable differences were found in the production of carbon dioxide by the roots of different species of plants.
7. Consideration of the relative size and area of root systems in connection with CO_2 output leads to the conclusion that carbon dioxide is an important factor in aiding certain kinds of plants to secure essential elements from the soil.
8. The production of carbon dioxide, whether from the standpoint of unit of dry matter of roots or from that of unit area of root system, as estimated in these experiments, has been greater for buckwheat and rye than for barley and corn.
9. The results are in general agreement with practical experience as to the ability of plants to grow satisfactorily on infertile soils.

The writer desires to acknowledge his indebtedness to Dr. C. A. SHULL and Dr. S. V. EATON of the Department of Botany for their kindly interest and many helpful suggestions throughout the course of the experiments.

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THE HEMISPHAERIACEAE OF BRITISH GUIANA AND TRINIDAD

F. L. STEVENS AND H. W. MANTER

(WITH PLATES XVIII-XXI)

The Hemisphaeriaceae, or "fly-speck" fungi, are chiefly tropical and are strictly superficial upon the surface of leaves. They either lack a true mycelium or it is so inconspicuous as to escape observation. The appearance of the flattened, circular, usually blackened thyriothechia (perithecia) on the leaf has led to the popular term "fly-specks." The studies in the present paper are based on material collected by the senior author in British Guiana and Trinidad during the summer of 1922. General observations in regard to the collecting, together with a map of the regions covered, as well as a list of the field numbers with places and dates of collection may be found in a previous paper (10). The original slides, drawings, and specimens upon which these studies are based are deposited in the herbarium of the University of Illinois. The photographs in the present paper are by A.G. ELDRIDGE.

The general typical appearance of the thyriothechia spotting the leaf surface is shown in figs. 1 and 3. The central region of old thyriothechia sometimes falls away, giving the appearance shown in fig. 2. These disclike bodies are easily removed from the leaf, and are readily studied in temporary water mounts, where the fruit body can be cut or crushed to reveal its asci and spores. Light staining with iodine may be advantageous. Permanent mounts of the thyriothechia are easily made by the celloidin method (9). The thyriothechia of the most common group of these fungi are carbonaceous and opaque, except for a narrow peripheral region which is blue-green; others may be brown in color. Old thyriothechia often appear entirely carbonaceous. The extremely variable and beautiful conditions of the spores and asci lend especial interest to the group. The taxonomy of the group is somewhat confused, and some history of the changes it has undergone is important.

The Hemisphaeriaceae are very similar to the Microthyriaceae. The latter is a very large group of some 32 genera (of which *Asterina* alone contains over 100 species), also chiefly tropical in distribution, and likewise possessing dislike, superficial thyriothecia. In fact, the two groups were not distinguished for a long time, both being united under the Microthyriaceae.

Apparently the first attempt to separate the blue-green "fly-specks" from the Microthyriaceae was made by VON HÖHNEL in 1910 (22). In commenting upon *Micropeltis applanata*, he pointed out that this species differed from the ordinary species of the Microthyriaceae in that the thyriothecia were not formed as inverse fruit bodies. He accordingly listed *Micropeltis* and *Scolecopeltis* as disc-shaped Sphaeriaceae. In 1912 (23), he discarded from *Micropeltis* many of the former species, on the basis of his former distinction between them and the Microthyriaceae. He stated that many of the so-called *Micropeltis* species, especially the smaller forms with radial structure, belong to the Microthyriaceae proper. Before that time 69 species of *Micropeltis* had been described. Of these, VON HÖHNEL retained only 23 as true *Micropeltis* species, and assigned the others to various genera among the Microthyriaceae and lichens.

In 1913 THEISSEN (16) established a distinction between the Microthyriaceae and the group represented by *Micropeltis* by erecting a new family, Hemisphaeriaceae, for the latter type; the Microthyriaceae being characterized by inverse and radiate thyriothecia, the Hemisphaeriaceae by non-inverse and non-radiate thyriothecia. Furthermore, THEISSEN united all the species with disc-shaped thyriothecia into a new order, which he named Hemisphaerales (16). In this order, in addition to the previously mentioned families, he included the Trichopeltaceae, which possess disc-shaped thyriothecia but also a vegetative thallus. A key to these families superficial on the host would then be (according to THEISSEN and SYDOW):

Thyriothecal membrane radial

Thallus thready or lacking.....Microthyriaceae

Thallus membranous, radial.....Trichopeltaceae

Thyriothecal membrane not radial.....Hemisphaeriaceae

THEISSEN then proceeded to divide the Hemisphaeriaceae into subfamilies based on the structure of the thyriothecal membrane.

The Dictyopeltineae (Dictyopelteae) possess a netlike covering blue-green or blue-black in color, and the Thrausmatopeltineae (Thrausmatopelteae) a pseudo-parenchymatic covering, easily breaking into pieces and yellow to brown-black in color. A third subfamily later named by THEISSEN (17) is Plochmopeltineae, with thyriothelial membrane of meandering, plectenchymatic structure, and with a netlike mycelium without hyphopodia. STEVENS and GUBA have added a fourth subfamily (11), with "thallus that of the Thrausmatopeltineae, asci solitary, naked, without a covering membrane."

Key to subfamilies of Hemisphaeriaceae (modified after THEISSEN and SYDOW):

- Thyriothelial membrane open netted, blue-green, without true myceliumDictyopeltineae Theiss.
- Thyriothelial membrane pseudo-parenchymatic, brown, without true mycelium
 - Asci coveredThrausmatopeltineae Theiss.
 - Asci nakedGymnopeltineae Stevens and Guba
- Thyriothelial membrane meandering-plectenchymatic, mycelium netlike, no hyphopodiaPlochmopeltineae Theiss.

The Dictyopeltineae are by far the most common, with *Micropellis* as perhaps the most common genus. As has been shown, this genus in the past has been overworked as a pigeon hole for many fungi of the general characteristics of the group. Now, however, it has been conveniently divided into a number of genera. THEISSEN's step in placing all radiate thyriothecia in the Microthyriaceae was perhaps the most valuable delimitation.

As early as 1889, SPEGAZZINI (5) established the genus *Scolecopeltis*, which is distinguished from *Micropellis* chiefly in possessing filiform, many-septate spores which tend to fall apart into their component cells. This distinction is not entirely satisfactory, as it requires an arbitrary decision as to what constitutes a filiform spore. The many-septate condition of the spore is highly variable in spores of more than a few cells. The loose connection between the cells in the spore, considered together with the filiform character, however, makes *Scolecopeltis* quite definitely distinct from *Micropellis*. The type species of *Scolecopeltis* is aparaphysate. It has seemed best in this paper to erect for all species which otherwise agree with

Scolecopeltis, but which possess paraphyses, a new genus, *Scolecopeltidium*.

The presence or absence of paraphyses seems to be a constant character, and a valid distinction upon which to separate genera. In 1913 SYDOW (12) utilized this character to distinguish a new genus, *Micropeltella*, without paraphyses, from *Micropeltis*, the type of which possesses paraphyses. *Micropeltella* at once adopted many of the old species of *Micropeltis*.

When the number of cells in the spores of these fungi is small, it usually is a constant character; hence, spore septation has been used to separate a few genera from *Micropeltis*. Those with 1-celled spores were allotted to *Dictyothrina* by THEISSEN (16); 2-celled spores to *Dictyothyrium* by THEISSEN (14); and 3-celled spores to *Dictyothyriella* by REHM (17). This last genus includes some of the most common members of the group, and numerous previously described *Micropeltis* species. *Dictyopeltis* Theiss. (16), with 2-celled spores, is distinct from *Dictyothyrium* in that it has no ostiole, but when ripe the whole thyriothecium breaks away from its rim.

Recently, SPEGAZZINI has separated several genera on the basis of the possession of stellate ostioles instead of the normal circular ostioles. The three genera created on such a basis are:

STELLATE OSTIOLE	ROUND OSTIOLE
<i>Micropeltidium</i>	separated from..... <i>Micropeltis</i> (6)
<i>Parapeltella</i>	separated from..... <i>Micropeltella</i> (6)
<i>Scolecopeltella</i>	separated from..... <i>Scolecopeltis</i> (7)

It seems somewhat doubtful whether this stellate or round condition of the ostiole is a sufficiently definite character to be of taxonomic value. In all the material observed in the present studies, none of the thyriothecia appeared to possess what could be called a characteristically stellate ostiole. It is true, however, that ostioles become quite irregularly circular, due to a tendency of their borders to fray or slightly split (figs. 55-57). If the very definite stellate condition illustrated by SPEGAZZINI (6) is a constant and permanent form, then it may well be considered as a valid and useful distinction. Since no specimens of such a type were seen, these three genera of SPEGAZZINI are accepted and included in the key to the genera of the

Dictyopeltineae. Curiously enough, SPEGAZZINI, in his key to the Dictyopeltineae (7), lists his genus *Parapeltella* as with a round ostiole, and separates it from *Micropeltella* by its clavate spores, a new distinction which hardly seems justified. Since the type description of the genus holds validity, this later discrepancy is doubtless due to an error in the publication of his key.

There appears to be another point of confusion in the consideration of SPEGAZZINI's genus *Micropeltidium*. As noted, this genus was separated from *Micropeltis* in 1919 only on the basis of its stellate ostiole. In the original description the genus was characterized as with paraphyses, and the type species (*Micropeltis tonduzii*) is densely paraphysate. It seems clear, then, that the presence of paraphyses is a character of the genus, as, indeed, otherwise it would correspond with *Parapeltella*. In 1923, however, SPEGAZZINI (7) seems to describe *Micropeltidium* again as a new genus, and here he points out that it is aparaphysate. The two species listed under it in 1923 are aparaphysate. Accepting the earlier and type descriptions of the genera involved, these two species seem to belong to *Parapeltella*.

This confusion in the conception of *Micropeltidium* has involved also another genus, *Metapeltella*, which SPEGAZZINI named in 1923 (7) in connection with his genus *Micropeltidium*, named at that time. *Metapeltella* is like *Micropeltidium*, except that the spores are clavate with the tip cell much larger. Since the genus is based on SPEGAZZINI's second *Micropeltidium*, *Metapeltella* is aparaphysate with stellate ostiole. SPEGAZZINI himself remarks that the spores of *Metapeltella* are like those of *Parapeltella*. As has been shown, the second *Micropeltidium* could not be distinguished from SPEGAZZINI's earlier *Parapeltella*, and this agreement of spore shape makes the identity of the two genera (*Metapeltella* and *Parapeltella*) even more perfect. From a careful study of the descriptions of these genera, it does not seem possible to separate *Metapeltella* from *Parapeltella* (as originally described), while the second *Micropeltidium* is separated from *Parapeltella* only on the basis of its possession of fusoid instead of clavate spores, and from *Micropeltella* only by its stellate ostiole. Whether genera should be separated on such considerations seems doubtful, but as no material of these species or groups was encountered in the

material examined by us, the genera are included in the following key. SPEGAZZINI's second *Micropeltidium* is designated as *Micropeltidium* no. 2.

One other puzzling point remains in regard to SPEGAZZINI's genus *Micraspidium*. No reference could be found to this genus except in SPEGAZZINI's key to the Dictyopeltineae in 1923. According to this key, *Micraspidium* cannot be distinguished from the earlier *Micropeltidium*, which, of course, is not represented in the key except as the second *Micropeltidium* (of different characters). Without knowledge of the type species of *Micraspidium* no definite statement can be made, but it may be significant that if the earlier *Micropeltidium* had been published as *Micraspidium*, most of the seeming contradictions in SPEGAZZINI's 1923 paper would be cleared up.

A genus, *Micropeltopsis*, described by EARLE¹, introduces a new distinguishing character. *Micropeltopsis* is separated from *Micropeltis* by possessing a white membranous border or halo around the thyriothecia. There are two objections to such a separation. First, the present studies seem to show that the halo, sometimes prominent, may be reduced in old thyriothecia of the same species, and in many cases even when present it can easily be lost in removing the thyriothecia from the leaf. Second, it is a character rarely mentioned in old descriptions, and the whole group would be of uncertain position until re-examined. *Micropeltopsis*, therefore, is not recognized in the following key.

All of these genera of the Dictyopeltineae are characterized by a netlike thyriothecial covering, and also by a blue-green color. THEISSEN in 1913 (15) noted this correlation, although the important character is the type of structure. The blue-green color is given by THEISSEN as accompanying all of the Dictyopeltineae known. One form was found in the present studies, however, which clearly possessed a coarse, open netted thyriothecial covering, but was a rich brown. This form is included under the Dictyopeltineae as a new genus, *Theciopeltis*.

¹ SPEGAZZINI (7) credits EARLE with describing this genus with the characters noted, but no trace of the original description could be found. The genus is also unknown at the New York Botanical Gardens, where most of the EARLE's specimens are deposited.

Following generic characters as outlined, a key to the genera of the Dictyopeltineae (based on the key by THEISSEN and SYDOW 19) is given as follows:

- Thyriothelial membrane open netted
 - Thyriothelial membrane blue-green
 - Spores 1-celled.....*Dictyothyria* Theiss.
 - Spores 2-celled
 - Ostiole present.....*Dictyothyrium* Theiss.
 - Ostiole absent.....*Dictyopeltis* Theiss.
 - Spores 3-celled.....*Dictyothyriella* Rehm
 - Spores 4 to many-celled
 - Paraphyses present
 - Ostiole round.....*Micropeltis* Mont.
 - Ostiole stellate.....*Micropeltidium* Speg.
 - Paraphyses absent
 - Ostiole round.....*Micropeltella* Syd.
 - Ostiole stellate
 - Spores clavate.....*Parapeltella* Speg.
 - Spores fusoid.....*Micropeltidium* no. 2 Speg.
 - Spores filiform
 - Paraphyses present.....*Scolecopeltidium* Stevens and Manter
 - Paraphyses absent
 - Ostiole round.....*Scolecopeltis* Speg.
 - Ostiole stellate.....*Scolecopeltella* Speg.
 - Thyriothelial covering brown.....*Theciopeltis* Stevens and Manter

Each genus represented in the material studied will be considered later, together with keys to the species.

Dictyothyrium Theiss. Oster. Bot. Zeitschr. 62:277. 1912

This genus has only a few species. It is clearly separated from *Micropeltis* which it resembles except that it has 2-celled spores. *M. corruscans* Rehm (3) is described as having 1-septate spores, so that we consider this species also as *Dictyothyrium*.

- Spores clavate.....*D. subcyaneum* (Ellis and Mart.) Theiss.
- Spores cylindrical
 - Paraphyses present
 - Asci 2-spored.....*D. disporum* Stevens and Manter
 - Asci with variable number of spores
 - Epiphyllous
 - Asci 90 μ in length.....*D. chalybaeum* (Rehm) Theiss.
 - Asci 125-170 μ*D. leucopterum* Syd.
 - Mostly hypophyllous.....*D. giganteum* Syd.
 - Paraphyses absent
 - Asci 8-spored.....*D. corruscans* (Rehm), n. comb.

1. *Dictyothyrium disporum* Stevens and Manter, n. sp. (fig. 8)
—Thyriothecia amphigenous, mostly hypophyllous, 535 μ in diameter, margin blue-green; ostiole round, up to 40 μ in diameter; asci obclavate, stipitate, 138 \times 33 μ , always 2-spored; paraphyses filamentous, crooked, copious; spores large, cylindrical, sometimes somewhat pointed at ends, especially when young, obtusely rounded at ends when mature, 1-septate, constricted, cells usually equal, basal cell sometimes slightly longer, spores 70–95 \times 10–12 μ .

On unknown member of the Menispermaceae, British Guiana: Rockstone, July 16, 435.

This fungus is nearest *D. leucopteryum* Syd., but differs from it in being mostly hypophyllous and in always having 2-spored asci. It differs from *D. giganteum* Syd. by having larger and 2-spored asci.

2. *DICTYOTHYRIUM GIGANTEUM* Syd. Phil. Jour. Sci. 9:C. 178.
1914

On *Serjania paucidentata*, British Guiana: Kartabo, July 23, 586.

This fungus agrees with the preceding species in all measurements and in spore septation. It disagrees, however, in a number of characters. Thus, asci were found with 1–3–4–6–8 spores. Great variation was found in the size and appearance of spores in the same ascus. Owing to the small amount of material available, the fungus has been accorded to the species *D. giganteum*, which it most closely resembles. A study of further material, if possible, would perhaps establish it as a new species.

3. *DICTYOTHYRIUM LEUCOPTERUM* Syd. Ann. Myc. 14:93. 1916

On *Eperua* sp., British Guiana: Wismar, July 14, 271.

DICTYOTHYRIELLA Rehm, Broteria 12:92. 1914

Dictyothyriella is perhaps of more frequent occurrence than any other genus in the group. Its 2-septate spores are quite constant, and separate it from *Micropeltis*. BEELI (1) does not seem to recognize *Dictyothyriella*, as he describes two *Micropeltis* species (*M. wil-demanii* and *M. dubia*) with 2-septate spores. We are including these species in the key under *Dictyothyriella*. The genus is also apparently accidentally omitted from SPEGAZZINI's key to the *Dictyopeltineae* in 1923.

Members of the same species of *Dictyothyriella* seem to grow on a great variety of hosts. Not only is the same species found on widely different hosts, but the same species appears to occur in widely differ-

ent parts of the world. This condition of host relationship and distribution seems to be characteristic of the Hemisphaeriaceae in general. While there may be biological specialization among the "fly-specks," as the fact that they are not found indiscriminately on all leaves in their neighborhood seems to indicate, it is not in many cases manifested morphologically.

Extreme morphological variation is often found within a single species of *Dictyothyriella*. As a result of this variation it is often very difficult to separate species, and some species necessarily embrace a large range of characters, and thus many examples of these species are found. For example, *D. heterosperma* has been found to exhibit nearly all possible variations, even within a single thyriothecium.

Species have been separated as distinctly as possible in the key, but it should be remembered that this key, as well as other keys in this paper, is sometimes forced to resort to characters of rather weak significance. The keys are entirely artificial. It is hoped that they will prove a convenient guide in determining species, but they cannot be considered as ultimate, nor even as bringing out the most important differences in every case. In a few cases no distinction could be discerned between two species from the descriptions, which are frequently inadequate. In such cases the two species are listed together.

Paraphyses lacking

Asci 8-spored

Spores not constricted.....*D. microsperma* (Syd.), n. comb.

Spores only slightly constricted.....*D. wildemanii* (Beeli), n. comb.

Spores strongly constricted

Asci 60-75 μ long.....*D. harmsiana* (P. Henn.), n. comb.

Asci 85-120 μ long.....*D. camarinensis* (Syd.), n. comb.

Asci 2-4-8-spored

Spores rarely 3-septate, 65-75 μ*D. megasperma* (Syd.), n. comb.

Spores rarely 1-septate, 36-48 μ*D. dubia* (Beeli), n. comb.

Paraphyses present

Asci 8-spored

Spores not over 20 μ

Hypophyllous.....*D. biseptata* (v. Höhn.) Theiss.

Epiphyllous

Spores obclavate.....*D. microspora* (Speg.) Theiss.

Spores clavate or oblong

Asci 30-35 μ*D. bauginiae* (Rehm), n. comb.

Asci 45-55 μ*D. treviae* Syd.

D. semecarpi (Syd.), n. comb.

- Spores $35\ \mu$ in length.....*D. trimera* (Sacc.), n. comb.
 Spores $38-62\ \mu$ in length.....*D. macromera* (Syd.), n. comb.
 Asci 4-spored
 Tip cell of spore pointed.....*D. coerulescens* (Rehm), n. comb.
 Asci with variable number of spores
 Tip cell of spore pointed
 Asci usually 4 or 6-spored, rarely 2-spored
 Spores $30-60\ \mu$*D. guianensis* Stevens and Manter
 Asci usually 2-spored, sometimes 4.*D. philodendri* Stevens and Manter
 Asci 4-8-spored, usually 8, rarely 2
 Spores $50-72\ \mu$*D. heterosperma* Syd.
 Tip cell of spores not pointed
 Asci 2-4-6-spored
 Spores $60-90-100\ \mu$ long.....*D. vismiae* Stevens and Manter
 Asci 2-4-6-8-spored
 Spores $30-38\ \mu$ long.....*D. mucosa* Syd.

4. *Dictyothyriella guianensis* Stevens and Manter, n. sp. (figs. 10-13).—Thyriothecia epiphyllous, either gregarious or somewhat scattered, $590-850\ \mu$ in diameter, margin blue-green, netted; ostiole round, $25-45\ \mu$ in diameter; asci cylindrical, short stipitate, 4-6-8-spored, rarely 2-spored, usually 4-6-spored, $100-155 \times 15-25\ \mu$; paraphyses copious, filamentous, crooked; spores typically 2-septate, constricted, cells easily separating, tip cell always larger and pointed, $30-60 \times 5-8\ \mu$.

On *Costus* sp., British Guiana: Rockstone, July 16, 1905; on *Pesequeria latifolia*, British Guiana: Kartabo, July 22, 1934; on *Mauritia* sp., Trinidad: Cumuto, August 16, 1907; on *Philodendron* sp., British Guiana: Kartabo, July 23, 1906; on *Licenia* sp., *Tabernaemontana* sp., and *Bauhinia* sp., British Guiana: Rockstone, July 17, 1907, 1934, 1935; on unknown member of the Marantaceae, British Guiana: Kartabo, July 21, 1905; on unknown member of the Apocynaceae, British Guiana: Tumatumari, July 11, 1935; on unknown host, British Guiana: Kartabo, July 17-23, 1930, 1936, 1938; Tumatumari, July 8-11, 1933, 1935; Wismar, July 14, 1925.

This fungus showed considerable variation, and often tended to approach *D. heterosperma*, which it closely resembles, especially in spore shape. *D. guianensis*, however, is always epiphyllous (although this cannot be considered a very significant character), usually has less than eight spores in the ascus, and all measurements are usually less than those of *D. heterosperma*.

5. *Dictyothyriella philodendri* Stevens and Manter, n. sp. (figs. 24-26).—Thyriothecia epiphyllous, $960\ \mu$ in diameter, ostiole round, $25\ \mu$ in diameter; asci clavate to elongate, often slightly curved, stipi-

tate, $150-155 \times 17-18 \mu$, 2-4-spored; paraphyses filamentous, crooked, not longer than asci; spores 2-4-septate, deeply constricted, cells easily separating, sometimes separate in the ascus, terminal cell elongated-pyriform with narrow distal region, longer than other cells, spores $50-60 \mu \times 7-9 \mu$.

On *Philodendron* sp., British Guiana: Kartabo, July 23, 1906, 479.

This fungus is close to *D. guianensis*. It differs in possessing usually 2-spored asci, asci and spores more elongated, and spores frequently 3 or 4-septate.

6. *DICTYOTHYRIELLA HETEROSPERMA* Syd., Ann. Myc. 15:231. 1917 (figs. 14-23)

On *Coccoloba* sp., British Guiana: Kartabo, July 23, 1907; on unknown member of the Myrtaceae, British Guiana: Rockstone, July 17, 1886; on unknown member of the Anonaceae, with *Micropellella minima*, British Guiana: July 24, 1911; on unknown host, British Guiana: Kartabo, July 22-24, 1911, 600, 1003, 666.

In his original description, SYDOW pointed out the high degree of variability of this fungus. Some of these specimens showed an even greater degree of variability. Thyriothecia were occasionally amphigenous, but commonly hypophyllous. The asci contained almost any number of spores up to eight. It can be seen that numerous characters must be considered to separate this species from *D. guianensis*.

An unusual condition was observed in one of the specimens, where the ascus frequently had four or six spores. One ascus was found with three large spores and one spore near the tip considerably dwarfed (fig. 16). The very marked difference between the size of this single spore and the others occupying the ascus seems to give a clue to the variability of the number of spores in the asci of this group, since it appears that some spores fail to develop, and finally degenerate, owing perhaps to competition among the spores in a single ascus.

Specimen no. 600 (figs. 18-22) revealed a remarkable variation, not only in spore septation, but also in the number of spores in the ascus. Thus in a single thyriothecium, one ascus contained four spores all many-septate (4-6-celled), another ascus had only three spores one of which was 4-celled, and another ascus had eight spores of normal septation. One ascus was found with only one spore, a number of asci with two spores, while a 4 or 6-spored condition was most common. The measurements of all parts coincided perfectly with those of *D. heterosperma*. Fortunately, specimens of this species identified by SYDOW were available for comparison. The principal difference noted was in the number of spores in the asci. In the authentic *D. heterosperma*, either six or eight spores were almost invariably present. This difference, however, does not seem to be sufficient to exclude the present specimens, and they are accordingly identified as *D. heterosperma*. Associated with this fungus (no. 600) was *Micropellella minima*.

7. *Dictyothyriella vismiae* Stevens and Manter, n. sp. (figs. 27-29).—Thyriothecia epiphyllous, 600-700 μ in diameter, margin blue-green; ostiole round, 40 μ in diameter; asci obclavate to cylindrical, sessile or very short stipitate, 4-6-spored, paraphyses copious, filamentous, crooked; asci 140-160 \times 20-35 μ ; spores 2-septate, deeply constricted, cells nearly equal and easily separating, tip cell not pointed, cells nearly oval in shape, spores 60-90 \times 12-16 μ .

On *Vismia* sp., British Guiana: Rockstone R. Ry., July 15, 1914; on *Coccoloba* sp., British Guiana: Rockstone, July 17, 1916; on unknown member of the Loranthaceae, British Guiana: Kartabo, July 21, 1916; on unknown host, British Guiana: Kartabo, July 23, 1916.

This fungus differs from *D. heterosperma* and *D. guianensis* in having all the cells of the spore nearly equal, and with the tip cell bluntly rounded instead of pointed. Material from nos. 1916, 1916, and 1916 sometimes showed spores so large that these numbers were at first allotted to a new species. As in all other respects they were similar to *D. vismiae*, and as even spore measurements frequently agreed, they are included in that species even though some of the spores were 50-150 μ in length and asci sometimes 250 μ long.

MICROPELTIS Mont., Pl. Cell. de L'île de Cuba, p. 325. 1842

The following species of *Micropelletis* are described having thyriothecia with radial coverings, and are therefore considered as excluded from the Hemisphaeriaceae: *M. sirie* Rac., *M. bambusae* Pat., *M. heteropteridis* Theiss. The two following species are inadequately described: *M. stigma* Cooke, *M. asterophora* B. and Br. Species described as without paraphyses are considered as belonging to *Micropelletella*.

Thyriothecia blue-green

Spores with constant number of septa

Spores with 3 septa

Asci 4-spored.....*M. tetraspora* Stevens and Manter

Asci usually 8-spored

No true ostiole.....*M. pitya* Sacc.

Ostiole present

Asci 6-8-spored.....*M. marginata* Mont.

Asci 8-spored

Hypophyllous.....*M. albo-ostiolata* P. Henn.

Epiphyllous

Asci clavate..... $\left\{ \begin{array}{l} M. depressa \text{ Cke.} \\ M. oleandri \text{ Br. and Har.} \end{array} \right.$

Asci oblong.....*M. pomeliae* Rehm

Amphigenous.....*M. vagabunda* Speg.

8. *Micropeltis tetraspora* Stevens and Manter, n. sp. (fig. 30).—Thyriothecia sparse, hypophyllous, $585\ \mu$ in diameter, margin blue-green, surrounded by transparent halo; ostiole unevenly round, $40\ \mu$ in diameter; asci cylindrical to subclavate; paraphyses copious, filamentous; asci always 4-spored, $146\text{--}187 \times 37\ \mu$; spores 3-septate, deeply constricted, cells loosely connected, basal cell elongated; spores $93\text{--}133 \times 13\text{--}16\ \mu$.

On unknown host, British Guiana: Kartabo, July 24, 1953.

This fungus differs from *M. albo-ostiolata* in having 4 instead of 8-spored asci, and in greatly different spore measurements.

In general appearance and measurements it resembles *M. dissociabilis*, but this latter fungus has 6–8-spored asci and 3–4–5-septate spores.

9. *Micropeltis dispora* Stevens and Manter, n. sp. (figs. 31–33).—Thyriothecia amphigenous, $817\ \mu$ in diameter, border blue-green; ostiole round, $40\text{--}48\ \mu$ in diameter; asci cylindrical, short stipitate, $160 \times 26\ \mu$, always 2-spored, paraphyses copious, short, crooked, filamentous; spores 3–4-septate, very rarely 5-septate, constricted, $90\text{--}103 \times 8\text{--}13\ \mu$.

On unknown host, British Guiana: Kartabo, July 23, 1953.

This fungus is interesting in the constant 2-spored condition of the mature ascus, and in the variation in spore septation. Thus three asci were found in which one of the two spores was 3-septate and the other 4-septate; one ascus in which both spores were 4-septate; one ascus in which both spores were 3-septate; and one ascus was found with one spore 5-septate. The fungus differs from neighboring species (*M. evonymi*, *M. rhopaloides*, and *M. dissociabilis*) in the 2-spored condition of the ascus, none of the other species ever possessing less than six spores in the ascus. It is perhaps closer to *M. guianensis*, but this latter species is distinct from it in having 3–4-spored asci, and also in quite different spore measurements.

10. *Micropeltis dissociabilis* Stevens and Manter, n. sp. (fig. 38).—Thyriothecia scattered, amphigenous, $535\text{--}700\ \mu$ in diameter, margin blue-green, open netted; ostiole round in mature thyriothecia; opening covered with thin membrane in young thyriothecia; asci cylindrical to clavate, sessile, 6–8-spored, $160\text{--}175 \times 38\ \mu$; paraphyses short, crooked, filamentous; spores 3–4–5-septate, usually 4-septate, deeply constricted, cells egg-shaped when nearly mature, ellipsoidal when mature, easily separating, end cells rounded, basal cell sometimes attenuated, $87\text{--}125 \times 12\text{--}15\ \mu$.

On unknown host, British Guiana: Coverden, August 8, 1785.

This fungus differs from *M. rhopaloides* in shape of spores. It differs from *M. evonymi* in not having typically 3-septate spores, in number of spores in the ascus, and in having larger spores and asci; from *M. aroidicola* in size of parts and in number of spores in the ascus. The fungus is of interest in showing a striking resemblance to the common species of *Dictyothyriella*, but differing from them in having 4-septate instead of 2-septate spores. It illustrates a possible close relationship between *Micropeltis* and *Dictyothyriella*.

11. *Micropeltis aroidicola* Stevens and Manter, n. sp. (figs. 34, 35).—Thyriothecia epiphyllous, 1070 μ in diameter, margin netted, blue-green; ostiole round, 15 μ in diameter; asci clavate to cylindrical, sessile, 2-4-spored, usually 2-spored, 80-95 \times 15-17 μ ; paraphyses copious, filamentous, crooked, 1.5 times length of ascus; spores 4-6-septate, usually 5-septate, constricted, cells loosely joined, tip cell spherical, basal cell often slightly elongated; spores 30-32 \times 10 μ .

On *Philodendron* sp., British Guiana: Kartabo, July 23, 544.

This fungus differs from *M. rhopaloides* and *M. borneensis* in shape of the spore and in the number of spores in the ascus. *M. aeruginascens* also has a different spore shape and more spores in the ascus. *M. dispersa* has a much larger spore measurement.

12. *Micropeltis guianensis* Stevens and Manter, n. sp. (fig. 36).—Thyriothecia epiphyllous, scattered, 750 μ in diameter, ostiole round, 45-50 μ in diameter; asci clavate to cylindrical, shortly stipitate, 125-140 \times 15-25 μ , apex blunt, stipe 3-8 μ , 3-4-spored; paraphyses copious, filamentous, crooked; spores 4-5-septate, constricted, hyaline, inordinate, 50-65 \times 10 μ .

On unknown host, British Guiana: Tumatumari, July 12, 247.

It differs from *M. samarensis* Syd. in having 4-5-septate instead of 6-7-septate spores, also in spore shape; from *M. rhopaloides* Syd. in spore shape and size.

13. *MICROPELTIS RHOPALOIDES* Syd., Ann. Myc. 15:230, 1917 (fig. 37)

On unknown member of the Bignoniaceae, British Guiana: Penal Settlement, July 25, 683.

This fungus differs from the original description only in being epiphyllous instead of amphigenous, and in having slightly larger thyriothecia.

MICROPELTELLA Syd., Ann. Myc. 11:404, 1913

All *Micropelletis* species described as aparaphysate are included in the following key. EARLE described *M. longispora* as without paraphyses, but as the thyriothecial membrane is dark brown, it cannot belong to either *Micropelletis* or *Micropellotella* and is omitted from the key.

Spores with not more than 3 septa

Ascus 5-6-spored.....*M. exilis* (Schulz.), n. comb.

Ascus 8-spored

Spores with 2-3 septa.....*M. bogoriensis* (v. Höhn.) Syd.

Spores with 3 septa

Ascus obclavate.....*M. minima* Stevens and Manter

Ascus fusoid

Ascus 36-40 μ*M. leucoptera* (P. and S.) Syd.

Ascus 45-55 μ*M. aequalis* (Sacc.), n. comb.

Ascus 85 μ*M. immarginata* (Rehm), n. comb.

Spores 3-4-septate

Ascus usually 8-spored..... $\left\{ \begin{array}{l} M. consimilis \text{ Rehm.} \\ M. merillii \text{ Syd.} \end{array} \right.$

Spores 4-septate

Ascus 180-200 μ*M. maxima* Speg.

Ascus 70 μ*M. cauma* (Speg.), n. comb.

Spores 4-5-septate

Spores not constricted.....*M. acensis* Syd.

Spores constricted

Hypophyllous.....*M. clavisporea* Syd.

Epiphyllous.....*M. constricta* Stevens and Manter

Spores 5-6-7-septate

Ascus 8-spored.....*M. agusanensis* Syd.

Ascus 4-6-8-spored

Epiphyllous, spores 5-7-septate.....*M. makilingiana* Syd.

Hypophyllous, spores 5-6-septate.....*M. ramosii* Syd.

Spores 6-many septate, asci 4-8-spored.....*M. paetensis* Syd.

Spores 7-septate, asci 8-spored.....*M. macropella* (P. and S.) Syd.

Spores 7-septate, asci 2-8-spored.....*M. sparsa* Stevens and Manter

Spores 7-9-septate, asci 8-spored.....*M. leptosphaerioides* Speg.

M. uleana (Syd.) and *M. albo-marginata* (Speg.) Syd. are too insufficiently described to be definitely placed in the key. *M. uleana* appears to be very near *M. immarginata*, and *M. albo-marginata* seems close to *M. consimilis*.

14. *Micropellotella minima* Stevens and Manter, n. sp. (fig. 39).—Thyriothecia epiphyllous, 259-320 μ in diameter, margin

blue-green, netted, ostiole round, $20\ \mu$ in diameter; asci obclavate, flask-shaped, 8-spored, paraphysate, $40\text{--}55 \times 20\ \mu$; spores 3-septate, $15\text{--}17 \times 3\text{--}5\ \mu$, both ends rounded, cells nearly spherical.

On unknown member of the Anonaceae, British Guiana: Tumatumari, July 9, 1902; Kartabo, July 24, 1912; on unknown host, British Guiana: Kartabo, July 23, 1910.

This fungus is near *M. albo-marginata* and *M. leucoptera*. It differs in shape and size of asci, and in being constantly 3-septate. Specimen no. 1010 differed from the type species only in having larger thyriothecia (up to $468\ \mu$). With it was associated *Dicthythiella heterosperma*.

15. *MICROPELTELLEA ACRENSIS* Syd., Ann. Myc. 14:89. 1916

On unknown member of the Celastraceae, British Guiana: Rockstone, July 15, 1910.

16. *Micropeltella constricta* Stevens and Manter, n. sp. (figs. 40-42).—Thyriothecia epiphyllous, scattered, $320\text{--}450\ \mu$ in diameter, margin blue-green, only slightly carbonaceous, ostiole round, $25\text{--}35\ \mu$ in diameter; asci cylindrical to obclavate, sessile, paraphysate, $75\text{--}88 \times 17\text{--}25\ \mu$, 6-8-spored; spores 3-6-septate, usually 4-5-septate, tip cell bluntly rounded, $25\text{--}45 \times 5\text{--}10\ \mu$, constricted.

On *Coccoloba* sp., Trinidad: Cumuto, July 16, 1914; on unknown member of the Anonaceae, British Guiana: Kartabo, July 22, 1910.

This fungus agrees with *M. acensis* Syd. except in having plainly constricted spores. The latter specimen (no. 568) differed from the type species in the size of the thyriothecia. Here they almost always measured about $935\ \mu$ in diameter and were mostly carbonaceous. Note should also be made of the deceptive appearance of empty asci which resemble paraphyses. Only careful examination shows that what appear to be straight paraphyses are in reality the edges of husklike, emptied, or degenerate asci. This is common in *Micropeltella*, and, in the examination of any material, structures which resemble any form of paraphyses other than the usual crooked, filamentous type, should be carefully studied.

17. *Micropeltella sparsa* Stevens and Manter, n. sp. (figs. 43, 44).—Thyriothecia epiphyllous, sparse, blue-green, only slightly carbonaceous, border membranous, transparent, thyriothecia $360\text{--}400\ \mu$ in diameter; ostiole irregularly round, $25\ \mu$ in diameter; asci sometimes visible through the thyriothecial covering, radially arranged about a non-ascigerous central region, cylindrical to fusoid, sessile, paraphysate, thick walled, bluntly rounded at each end,

80-100 \times 24-28 μ , 2-3-4-5-6-7-8-spored; spores fusoid-cylindrical, 6-7-8-septate, usually 7-8-septate, middle cells larger, tip cells bluntly rounded, slightly constricted, 36-48 \times 8-10 μ .

On *Anacardium occidentale*, British Guiana: Rockstone, July 13, 1913.

This fungus showed some points of resemblance to *M. paelensis* Syd., but differs in having much smaller thyriothecia with hyaline borders, and in number of spores in the ascus, as well as being on a widely different host.

Scolecopeltidium Stevens and Manter, n. gen.

Thyriothecia superficial, disc-shaped, true mycelium lacking, margin open netted, blue-green, ostiole round, spores filiform, many-septate, paraphyses present. Like *Scolecopeltis* except that paraphyses are present; like *Micropeltis* except that spores are filiform.

The type species of *Scolecopeltis* is aparaphysate, while about one-half of the described species possess paraphyses. In view of the fact that most of the new material encountered in the present study appeared to possess paraphyses, it has seemed convenient to separate such species by the erection of a new genus. The following former *Scolecopeltis* species fall into the new genus *Scolecopeltidium*: *S. salacensis* Rac., *S. gaduae* P. Henn., *S. guettardae* P. Henn., *S. trivialis* Rac., *S. bakeri* Syd., *S. connari* Syd., and *S. garciniae* Rehm.

Asci not more than 4-spored

Asci obclavate, spores up to 48-septate... *S. mirabile* Stevens and Manter

Asci cylindrical, spores 11-14-septate. *S. multiseptatum* Stevens and Manter
Asci 3-6-spored..... *S. hormosporum* Stevens and Manter

Asci 8-spored

Spores 6-7-septate..... *S. connari* (Syd.), n. comb.

Spores 6-12-septate..... *S. salacensis* (Rac.), n. comb.

Spores 10-12-septate

Hypophyllous, 4-8-spored..... *S. bakeri* (Syd.), n. comb.

Spores 13-16-septate

Epiphyllous, asci 160-175 μ *S. liciniae* Stevens and Manter

Spores 17-septate

Asci 120-130 μ *S. garciniae* (Rehm), n. comb.

Spores 14-20-septate..... *S. gaduae* (P. Henn.), n. comb.

Spores 15-23-septate

Epiphyllous..... *S. costii* Stevens and Manter

Spores 20-26-septate

Hypophyllous..... *S. guettardae* (P. Henn.), n. comb.

This key does not include *S. trivialis*, the spore septation of which could not be found in the description. It apparently resembles *S. salacensis*, differing in an ascus measurement of 149-165 \times 12 μ .

18. *Scolecopeltidium mirabile* Stevens and Manter, n. sp. (figs. 47-50).—Thyriothecia hypophyllous, 750-965 μ in diameter, margin wide, open netted, blue-green; ostiole round, minute, 15-20 μ in diameter; asci obclavate, sessile, 150 \times 40 μ , 2-4-spored; paraphyses short, filamentous, not crooked; spores filiform, many-septate (up to 48), constricted, variable, sometimes curled in ascus and twice as long as ascus; 115-125 \times 8-10 μ up to 275 \times 8-10 μ .

On unknown member of the Simarubaceae, British Guiana: Kartabo, July 23, 678.

19. *Scolecopeltidium multiseptatum* Stevens and Manter, n. sp. (figs. 51-53).—Thyriothecia epiphyllous, scattered, large, up to 1125 μ in diameter, ostiole round, 15 μ in diameter; asci cylindrical, sessile, broadly rounded at both ends, 125-138 \times 27-37 μ , 2-4-spored; paraphyses filamentous, crooked; spores filiform, 12-15-septate, constricted, each cell except the two end cells slightly constricted in the middle, straight or slightly curved, 100-120 \times 10 μ .

On *Philodendron* sp., British Guiana: Wismar, July 24, 1004; July 14, 264; Kartabo, July 22, 544.

20. *Scolecopeltidium hormosporum* Stevens and Manter, n. sp. (figs. 45, 46).—Thyriothecia epiphyllous, 1016-1070 μ in diameter, scattered, carbonaceous, very narrow blue-green margin; ostiole irregularly round, about 25 μ in diameter, not over 35 μ in diameter; asci clavate, sessile; paraphyses filamentous, slightly crooked, branched; asci either 3 or 6-spored, 175-195 \times 32-45 μ ; spores filiform, curved, many-celled, usually 25 or 26, immature spores of 6-spored ascus in 3 rows, mature spores often curved about one another, nearly as long as ascus, 165 \times 7-8 μ , tip cell slightly elongate, basal cell spherical and larger, other cells spherical, deeply constricted.

On unknown host, British Guiana: Kartabo, July 23, 581.

21. *Scolecopeltidium Liciniae* Stevens and Manter, n. sp. (fig. 54).—Thyriothecia epiphyllous, 500-600 μ in diameter, ostiole round, 25-35 μ in diameter; margin of thyriothecia open netted, blue-green; asci cylindrical, sessile or very short stipitate, 160-175 \times 45-48 μ , 8-spored; paraphyses filamentous, short, crooked, not copious;

spores filiform, 13-16-septate, not constricted, straight or slightly curved, $100 \times 10 \mu$.

On *Licenia* sp., British Guiana: Rockstone, July 17, 479; on unknown member of the Marantaceae, British Guiana: Rockstone, July 16, 423.

22. *Scolecopeltidium costi* Stevens and Manter, n. sp. (figs. 58-60).—Thyriothecia large, epiphyllous, blue-green, netted margin, 800-900 μ in diameter; ostiole round, up to 55 μ in diameter; asci cylindrical, little or no stipe, 8-spored, 175-200 μ long when mature and 37-45 μ wide; paraphyses copious, filiform, crooked; spores filiform, many-septate, 15 up to 23, constricted, straight or curved; young spores with fewer septa, young spores spindle-shaped at stage of 4-5-septa; mature spores with middle cells larger and rounded, longest spore $137 \times 12.5 \mu$.

On *Costus* sp., British Guiana: Rockstone, July 16, 425; on *Serjania paucidentata*, British Guiana: Kartabo, July 23, 585; on unknown host, British Guiana: Tumatumari, July 12, 234.

Specimen no. 585 differed from the type species in being hypophyllous. While the largest spore in the type species measured 137 μ , many spores apparently mature measured about 100 μ .

SCOLECOPELTIS SPEG., Bol. Acad. Cien. Cord. 11:196. 1889

No true specimens of this genus were found in the present studies, although forms with filiform spores and with paraphyses were quite numerous. As has been noted, these have been separated from *Scolecopeltis*, which is without paraphyses, and considered in the new genus *Scolecopeltidium*. Because the two genera are so closely related, a key of the described *Scolecopeltis* species is here included. It should also be recalled that a few very similar species, differing only in possessing "stellate" ostiole, have been described by SPEGAZZINI under *Scolecopeltella*. These species are not included in the key. Following THEISSEN and SYDOW, *Scolecopeltopsis* v. Höhn. is considered as synonymous with *Scolecopeltis*.

Asci 2-3-4-spored.....*S. aeruginea* Zimm.

Asci 8-spored

Spores 5-7-9-septate.....*S. transiens* (v. Höhn.) Sacc. and Trot.

Spores many-septate

Asci 75-100 μ (not over 100 μ)

Hypophyllous, spores 10-15-septate

Asci 20-25 μ wide.....*S. pachyasca* Speg.

Asci 42-45 μ wide.....*S. dissimilis* Rehm

Epiphyllous

Asci clavate.....*S. bauhiniae* P. Henn.

Asci at least 110 μ long

Asci 110 \times 40 μ , spores 40-septate.....*S. ophiospora* (Pat.) Sacc.

Asci 130-180 μ

Spores blunt, 15-septate.....*S. quindeciesepitata* P. Henn.

Spores pointed.....*S. tropicalis* Speg.

Asci 180-200 μ

Spores 15-20-septate.....*S. portoricensis* Speg.

RICK has listed a species, *Scolecopeltis theissenii* Rick, under the herbarium reference "exsicc. Rick, F. a. am. 250," but no printed description of the species or reference to publication could be found.

***Theciopeltis* Stevens and Manter, n. gen.**

Thyriothecia superficial, circular, disc-shaped, covering open netted, not radial, rich brown becoming opaque toward the center, border hyaline, no true ostiole, irregular stellate opening in ripe thyriothecia, little or no mycelium.

This genus is most like *Phragmothryiella* or *Microthryiella*, but differs quite markedly in the appearance of the covering of the thyriothecia, which is formed by a network of hyphae anastomosing or interweaving instead of the apparently cellular and easily disintegrating covering found in *Phragmothryiella* and *Microthryiella*. The breaking of the thyriothecia is at a definitely central point, while in *Phragmothryiella* and *Microthryiella* it occurs by a kind of fragmentation of the thin and quite transparent covering. On account of the netlike structure of *Theciopeltis* it is placed in the subfamily Dictyopeltineae, where it appears to be the first representative which is brown in color.

23. ***Theciopeltis guianensis*** Stevens and Manter, n. sp. (figs. 6, 7, 61).—Thyriothecia epiphyllous, 160-200 μ in diameter, circular, rich brown, widely open netted, border hyaline, no true ostiole, sometimes an irregular stellate opening; asci spherical (obclavate when mature), sessile, 20-30 μ , paraphyses few, slightly crooked, not branched; asci 6 or 8-spored; spores cylindrical, 1-2-3-septate, hyaline, 12-20 \times 4-5 μ .

On unknown host, British Guiana: Rockstone, July 17, 470.

THRAUSMATOPELTINEAE

The subfamily Thrausmatopeltineae proves somewhat more difficult, although the difficulties are chiefly connected with the species and genera. The subfamily itself is quite distinct in possessing thy-

riotheacial coverings of pseudo-parenchymatic tissue brown or yellow-brown in color. The individual cells in this covering are small and tend to be angular. Often, especially when an ostiole is absent, the covering breaks down into irregular pieces (as in *Microthyriella*). Hymenia (or ascigerous tissue), according to THEISSEN and SYDOW, may be one or many in the thyriotheacia, and each of these hymenia may bear one or many asci. These conditions of the hymenia are used by THEISSEN and SYDOW to separate wide groups of genera. Most forms occur in the group described as "only one hymenium present." These authors place here *Microthyriella*, although, as pictured by VON HÖHNEL (21), the asci in this genus are somewhat separated by hyphal growth ("pseudo-paraphyses"). Fortunately, separation of genera on the basis of the presence of "many hymenia" is rendered much less significant due to recent research. In this group, THEISSEN and SYDOW list three genera, each of but few species; *Polyclypeolum*, *Eremotheca*, and *Eremothecella*.

Eremotheca was described by THEISSEN and SYDOW in 1917 (19), who used *Rhytisma rufulum* B. and C. as the type, and named one new species, *E. philippinensis* Syd. In 1918, VON HÖHNEL (26) declared that the type species of *Eremotheca* is identical with *Microthyriella rickii* v. Höhn., and that *E. philippinensis* belongs in the same genus, differing from *M. rickii* only slightly in spore measurement. As a *Microthyriella philippinensis* had already been described, VON HÖHNEL called *E. philippinensis*, *M. macrospora*. Specimens of this fungus identified by SYDOW were available for study, and in every character seemed to agree with those of *Microthyriella*, so that we follow VON HÖHNEL in accepting *Microthyriella* as a synonym for *Eremotheca*.

Eremothecella Syd. (19) has but one species, *E. clamicola*. VON HÖHNEL (27) studied this and found it to be a lichen. It was identified by ZAHLBRUCKNER as belonging to *Arthoniopsis*. A cotype of this species was available for our study. It is evident that some lichens may be very closely similar in appearance to members of this group of the Hemisphaeriaceae. This particular specimen could very easily be mistaken as a member of the subfamily Gymnopeltineae Stevens and Guba (11), as there appears to be no covering over the asci.

Polyclypeolum has but a single species, *P. abietes* (v. Höhn.)

Theiss. VON HÖHNEL (25) expresses doubt that this genus can be separated from *Schizothyrium*.

All of the remaining genera of the Thrausmatopeltineae are more or less similar to *Microthyriella*, the most common genus. This genus was established in 1909 by VON HÖHNEL (21), with *M. rickii* Rehm as the type species. The covering of the thyriothecia is composed of small cells (4- or 5-sided), is brown, and lacks an ostiole. When mature the whole covering fragments, falling apart into irregular pieces. The spores are 2-celled.

Several other genera are so similar to *Microthyriella* that attention should be called to their exact position. At the time *Microthyriella* was described, VON HÖHNEL observed that it was like *Microthyrium* except that an ostiole was lacking. *Microthyrium*, however, is now considered by THEISSEN and SYDOW to belong in the Microthyriaceae, so that true *Microthyrium* species have a radial covering.

The spore septation, which is very constant here, easily separates *Haplopeltis* Theiss. (18) with 1-celled spores, and *Phragmothyriella* v. Höhn. (24) which has many celled spores. *Haplopeltis* has an ostiole, but *Phragmothyriella* is exactly similar to *Microthyriella* except for spore septation.

Clypeolum Speg. (4) is very similar to *Microthyriella*, as pointed out by VON HÖHNEL (22). The original description of *Clypeolum* gives it a carbonaceous thyriothecium and cylindrical asci, but VON HÖHNEL, in studying the type species, found the structure of the thyriothecium to agree with that of *Microthyriella*. He separated the two because of different shape of asci, and because *Clypeolum* possesses typical thready paraphyses, while the paraphyses of *Microthyriella* are branched and united into a tissue-like mass. On this basis, he allotted a number of former *Clypeolum* species to *Microthyriella*.

SPEGAZZINI, in 1923 (8), named a new genus, *Clypeolina*. This genus is like *Clypeolum* except that it has an ostiole. The name *Clypeolina* is preoccupied, however, as THEISSEN gave it to a genus of Microthyriaceae (14). We therefore propose the name *Clypeolopsis* Stevens and Manter for the genus *Clypeolina* named by SPEGAZZINI in 1923.

Schizothyrium Desm. (2) is also very similar to *Microthyriella*, as

shown by VON HÖHNEL (25). *Schizothyrium*, however, is not usually circular in form, but tends to be elongate (the type species measured 250–300 μ long by 180–200 μ wide). It has a thick, often carbonaceous membrane, instead of the thin one found in *Microthyriella*. It lacks an ostiole, but when ripe the thyriothechia open by means of a longitudinal split. The surface of the thyriothechia is sparsely covered with mycelial strands, which stream out over the angular cells of the membrane proper, and may extend slightly beyond the border.

A key to the genera of the Thrausmatopeltineae following the conclusions of this discussion follows.

One hymenium present

Spores 1-celled, colorless.....*Haplopeltis* Theiss.

Spores 2-celled, colorless

Thyriothechia round

Ostiole present.....*Clypeolopsis* Stevens and Manter

Ostiole lacking

Paraphyses present.....*Clypeolum* Spig.

Paraphyses lacking.....*Microthyriella* v. Höhn.

Thyriothechia elongated.....*Schizothyrium* Desm.

Spores many celled, colorless.....*Phragmothyriella* v. Höhn.

More than one hymenium present.....*Polyclypeolum* Theiss.

MICROTHYRIELLA v. HÖHN., SITZ. KAIS. ACAD. WISS. WIEN

118:371. 1909

REHM's former *Microphyma rickii* was taken by VON HÖHNEL as the type species for *Microthyriella*. The characters of the genus are well defined and have already been outlined. The relationships of the genus to *Clypeolum* and *Schizothyrium* have also been discussed.

Microthyriella is remarkable for the constancy of some characters which are notably variable among the Dictyopeltineae. For example, the asci are almost always ovoid-globose and sessile, always 8-spored; while the spores are constantly 1-septate. The large number of species are hence very largely separated on the size of parts. THEISSEN in 1914 (17) reviewed the genus up to that date, and lists fourteen species. Since then the number of species has considerably increased, and the genus is rather difficult to analyze.

Of the twenty-four species of *Microthyriella* now described, two possessed only immature spores, so their exact position cannot at

present be determined. These species are *M. celastri* (E. and K.) Th. and *M. perexigua* (Rob.) v. Höhn. To be listed as possible *Microthyriella* species are *Clypeolum sulcatum* Starb. and *C. megalosporum* Speg., both of which VON HÖHNEL (22) suggested as being probable *Microthyriella* species, although he did not rename them. Most of the other species which VON HÖHNEL suggested at the same time in a similar manner have since been definitely placed in the genus *Microthyriella*. In the absence of material for study, it has seemed best simply to point out that *C. sulcatum* might be near *M. uvariae* and *M. philippinensis*, although clearly distinct from them; and *C. megalosporum* might be near *M. rickii*. To these species are added two new species from British Guiana. THEISSEN (18) gives *M. olivacea* (v. Höhn.) Th. as a new combination for *Asterella olivacea* v. Höhn. VON HÖHNEL (27) later made the same change, apparently unaware that THEISSEN had already done so. The correct name therefore should read *Microthyriella olivacea* (v. Höhn.) Theiss.

As already noted, *Eremotheca philippinensis* Syd. becomes *Microthyriella macrospora* v. Höhn. *Microthyriella hibisci* Stevens and Guba (11) should perhaps be accorded a new genus, as the possession of an ostiole is not in agreement with the character of the genus.

- Ostiole present.....*M. hibisci* Stevens and Guba
 Ostiole absent
 Spores constricted
 Asci round
 Spores 8 μ long.....*M. intricata* (E. and M.) Th.
 Spores 37-47 μ long.....*M. guianensis* Stevens and Manter
 Asci ovate
 Spores 10 μ long.....*M. rimulosa* (Speg.) Th.
 Spores 28-30 μ long.....*M. pseudocuticulosa* (Winter) v. Höhn.
 Spores not or little constricted
 Asci pyriform
 Spores 9 μ*M. minutissima* (Speg.) Th.
 Asci spherical
 Asci 25 μ in diameter.....*M. cuticulosa* (Cke.) v. Höhn.
 Asci 27-35 μ in diameter.....*M. discoidea* (Cke.) Th.
 Asci ovoid or ellipsoid
 Spores not over 13 μ
 Asci with short stipe.....*M. astoma* (Speg.) The

often irregular in outline, covering membrane pseudo-parenchymatous, hymenium single, no ostiole; asci many, elongate, subcylindrical, ellipsoid, blunt at both ends, paraphysate, 8-spored, $50-75 \times 20-25 \mu$; spores colorless, 2-celled, ellipsoid, not or little constricted, not parallel, $12-13 \times 5-6 \mu$.

On unknown host, British Guiana: Kartabo, July 24, 1915.

The spores of this species are very small in relation to the size of the ascus. This proportion separates it from all other species in the genus.

PLOCHMOPELTINEAE

The Plochmopeltineae Theiss. (18) are separated from the other subfamilies of the Hemisphaeriaceae by possessing thyriothecial membranes of meandering, sinuous context, and also by possessing a free mycelium without hyphopodia. The thyriothecia are brown, although this character is not given in the subfamily description. Only a few genera are known. No representatives with mature spores were found in the present studies. MENDOZA, however, working at the University of Illinois, has described two new genera and a few new species in this group. These genera and species are included here, although a part of the material was collected in Hawaii. The descriptions of these forms are as given by MENDOZA, and the key to the genera is modified by him from THEISSEN and SYDOW.

Covering membrane without ostiole, paraphyses lacking

Spores 2-celled, hyaline

Covering membrane without setae.....*Plochmopeltis* Theiss.

Covering membrane with setae.....*Plochmopeltidella* Mendoza

Covering membrane ostiolate, hymenium with many asci

Spores 2-celled, hyaline

Covering membrane without setae

Paraphyses present.....*Stomiopeltis* Theiss.

Paraphyses absent.....*Stomiopeltella* Theiss.

Covering membrane with setae.....*Chaetopeltopsis* Theiss.

Spores 4-celled, hyaline

Covering membrane without setae

Paraphyses present.....*Scolecopeltidella* Mendoza

Plochmopeltidella Mendoza, n. gen.

Mycelium netlike, interwoven, with long cylindrical cells; thyriothecia without ostiole, covering membrane a winding prosenchyma, setose, with many asci, paraphysate; spores hyaline, 1-septate.

27. *Plochrompeltidella smilacina* Mendoza, n. sp. (figs. 64-66).—Mycelium of a netlike web, interwoven, with long cylindrical cells, straw colored to almost brown, branching, without hyphopodia; thyriothecia covered by a winding prosenchyma, without ostiole, setose, about $120\ \mu$ in diameter; setae few, acute, black, about $130\ \mu$ long and $9\ \mu$ wide near base, borne on thyriothecia and outgrowths from the long cylindrical cells; asci numerous, sickle-shaped to clavate, 8-spored, about $36 \times 12\ \mu$; spores more or less fusiform, hyaline, 1-septate, about $12 \times 4\ \mu$.

On *Smilax* sp., Oahu: Olympus, June 6, 670.

The characters of this fungus show it to be nearly related to *Plochrompeltis* Theiss., except in the presence of setae, which are not found in *Plochrompeltis*. Such difference is sufficient to warrant the erection of a new genus. The fungus is also associated with several other fungi, among them *Phragmocapnias smilacina*.

STOMIOPELTIS THEISS., Broteria 12:82. 1914

28. *Stomiopeltis cassiae* Mendoza, n. sp. (figs. 67, 68).—Mycelium irregularly branched, woven to weftlike, hyaline to honey-yellow; thyriothecia numerous, dimidiate, covering cells long, irregularly woven, yellowish to brown, ostiolate, about $100\ \mu$ in diameter; ostiole with definite border, about $7\ \mu$ in diameter; asci numerous, ovate, paraphysate, 8-spored, about $36 \times 9\ \mu$; spores ovate, hyaline, 1-septate, about $13 \times 3\ \mu$.

On *Cassia* sp., British Guiana: Tumatumari, July 10, 115.

This fungus is found in pure growth on the leaf, and appears as a leathery, thin, sooty mass on the upper surface. The colonies have no definite shape, and are more or less irregularly scattered, although they sometimes cover the whole surface. It is strictly epiphyllous.

Stomiopeltis aspersa (Berk.) Theiss., formerly called *Asterina aspersa*, and *Stomiopeltis rubi* (Fuck.) Petr., formerly called *Asterella rubi* (Fuck.) v. Höhn., are the only other species in this genus.

STOMIOPELTELLE THEISS., Broteria 12:85. 1914

29. *Stomiopeltella suttoniae* Mendoza, n. sp. (figs. 69-72).—Mycelium irregularly branched, gelatinous, honey-yellow to dark brown; thyriothecia numerous, dimidiate, covering cells long, irregularly woven and interwoven, with long cylindrical cells, yellowish to dark brown, ostiolate, gelatinous, about $120\ \mu$ in diameter; asci nu-

merous, ovate, paraphysate, 8-spored, about $44 \times 16 \mu$; spores ovate, hyaline, 1-septate, about $14 \times 5 \mu$.

On *Suttonia lessertiana*, Oahu: Honolulu, Hamakua upper ditch trail, July 28, 1932.

This fungus appears to agree fully with *Stomiopeltella*, of which *S. patagonica* (Speg.) Theiss. (formerly called *Microthyrium patagonica*) is the type species. It differs from the type species in having ovate instead of elongate asci and in wider spores. It differs from *S. nubecula* (B. and C.) Theiss. (the only other species in the genus) by having much smaller asci, which are ovate instead of cylindrical.

Scolecopeltidella Mendoza, n. gen.

Mycelium netlike, branching, hyaline; thyriothecia hemispherical, membranous, ostiolate, honey-yellow; asci 8-spored, paraphysate; spores hyaline, cylindrical, 3-septate.

30. *Scolecopeltidella palmarum* Mendoza, n. sp. (fig. 73-75).—Mycelium netlike, irregularly branched, hyaline to very pale straw-colored, radiating irregularly from the thyriothecia; thyriothecia membranous, honey-yellow, ostiolate, with indefinite border, about 168μ in diameter; asci numerous, ovate, paraphysate, 8-spored, about $36 \times 12 \mu$; paraphyses numerous, thready, spores hyaline, long-cylindrical, 3-septate, about $19 \times 4 \mu$.

On palm, British Guiana: Rockstone, July 17, 1942.

This fungus resembles *Scolecopeltis* in the general shape of the thyriothecia, the presence of an ostiole, and the shape and septation of the spores. It differs from *Scolecopeltis* in that it has paraphyses, 4-celled spores, a true superficial mycelium, and honey-yellow color. *Scolecopeltis* has many celled spores, blue-green thyriothecia, no true mycelium, and no paraphyses. The fungus appears as yellowish to almost brown specks, thinly scattered on both surfaces of the leaf. The thyriothecia are rare.

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EXPLANATION OF PLATES XVIII-XXI

Plate IV was copied from figures by Mr. JOSE MENDOZA; all other drawings were made by the aid of a camera lucida by Mr. MANTER.

PLATE XVIII

FIG. 1.—Leaf showing characteristic appearance of thyriothecia of fungus (*Micropelletella constricta*).

FIG. 2.—Enlarged portion of same.

FIG. 3.—Thyriothecia of *Dictyothyriella vismiae* on *Coccoloba* leaf.

FIG. 4.—General appearance of thyriothecia of *Dictyopeltineae*.

FIG. 5.—Portion of border of same enlarged to show netlike structure.

FIG. 6.—Thyriothecium of *Theciopeltis guianensis*.

FIG. 7.—Portion of border of same.

PLATE XIX

FIG. 8.—Asci and ascospores of *Dictyothyrium disporum*.

FIG. 9.—Asci and ascospores of *Dictyothyrium leucopteryum*.

FIGS. 10–13.—*Dictyothyriella guianensis*; asci and ascospores, showing variation.

FIGS. 14–17.—Asci and ascospores of *Dictyothyriella heterosperma*, showing variation in number of spores.

FIGS. 18–22.—Asci and ascospores of *Dictyothyriella heterosperma* from single peritheciium, showing great variability of spores.

FIG. 23.—Asci and ascospores of *Dictyothyriella heterosperma* from different host.

FIGS. 24–26.—Asci and ascospores of *Dictyothyriella philodendri*.

FIGS. 27–29.—*Dictyothyriella vismiae*; asci and ascospores.

FIG. 30.—Asci and ascospores of *Micropeltis tetraspora*.

FIG. 31.—Ascus, ascospores, and paraphyses of *Micropeltis dispora*.

FIG. 32.—Ascospores of same.

FIG. 33.—Ascus of same with spores.

FIGS. 34, 35.—Asci and ascospores of *Micropeltis aroidicola*.

FIG. 36.—*Micropeltis guianensis*; asci, paraphyses, and ascospores.

PLATE XX

FIG. 37.—Ascus and spores of *Micropeltis rhopaloides*.

FIG. 38.—Asci and spores of *Micropeltis dissociabilis*.

FIG. 39.—Asci and spores of *Micropeltella minima*.

FIG. 40.—Ascus and spores of *Micropeltella constricta*.

FIGS. 41, 42.—Asci and spores of same fungus from different host.

FIGS. 43, 44.—Asci and spores of *Micropeltella sparsa*.

FIGS. 45, 46.—Asci of *Scolecopeltidium hormosporum*, showing ascospores.

FIGS. 47–49.—Spores of *Scolecopeltidium mirabile*.

FIG. 50.—Spores of same in portion of ascus, showing position within ascus.

FIGS. 51–53.—Asci and spores of *Scolecopeltidium multiseptatum*.

FIG. 54.—Ascus and spores of *Scolecopeltidium liciniae*.

FIGS. 55–57.—Outline of ostioles of different thyriothecia on same leaf, showing variation (*Dictyothyriella vismiae*).

FIG. 58.—Asci of *Scolecopeltidium costi*, showing spores in very early stage of development.

FIG. 59.—Asci of same fungus showing spores at different stages of development.

FIG. 60.—Mature spores of *Scolecopeltidium costi*.

FIG. 61.—Ascospores of *Theciopeltis guianensis*.

FIG. 62.—Asci and spores of *Microthyriella guianensis*.

FIG. 63.—Asci and spores of *Microthyriella distincta*.

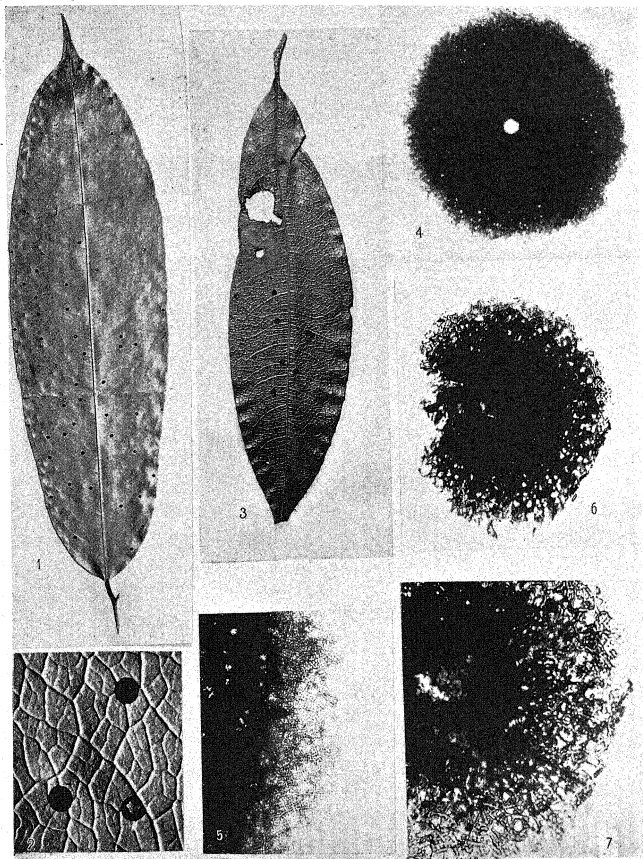
PLATE XXI

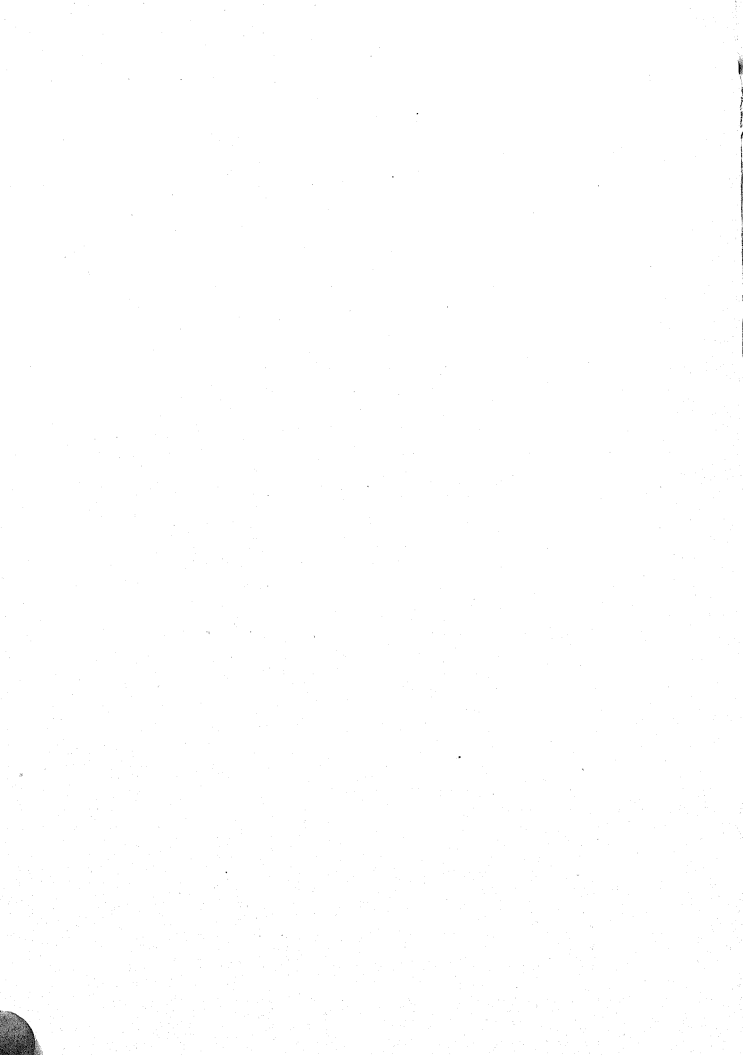
FIGS. 64-66.—*Plochrompeltidella smilacina*: fig. 64, thyriothecium showing character of covering membrane and portion of setae; fig. 65, two asci with ascospores; fig. 66, three ascospores showing sickle-shape to clavate form.

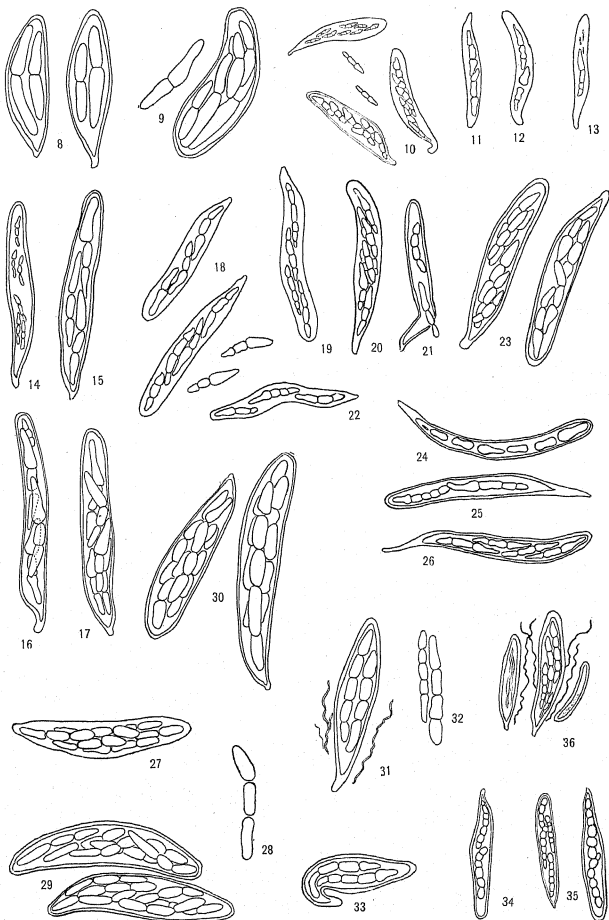
FIGS. 67-68.—*Stomiopeltis cassiae*: fig. 67, thyriothecium showing ostiole and asci with ascospores; fig. 68, ascus with ascospores.

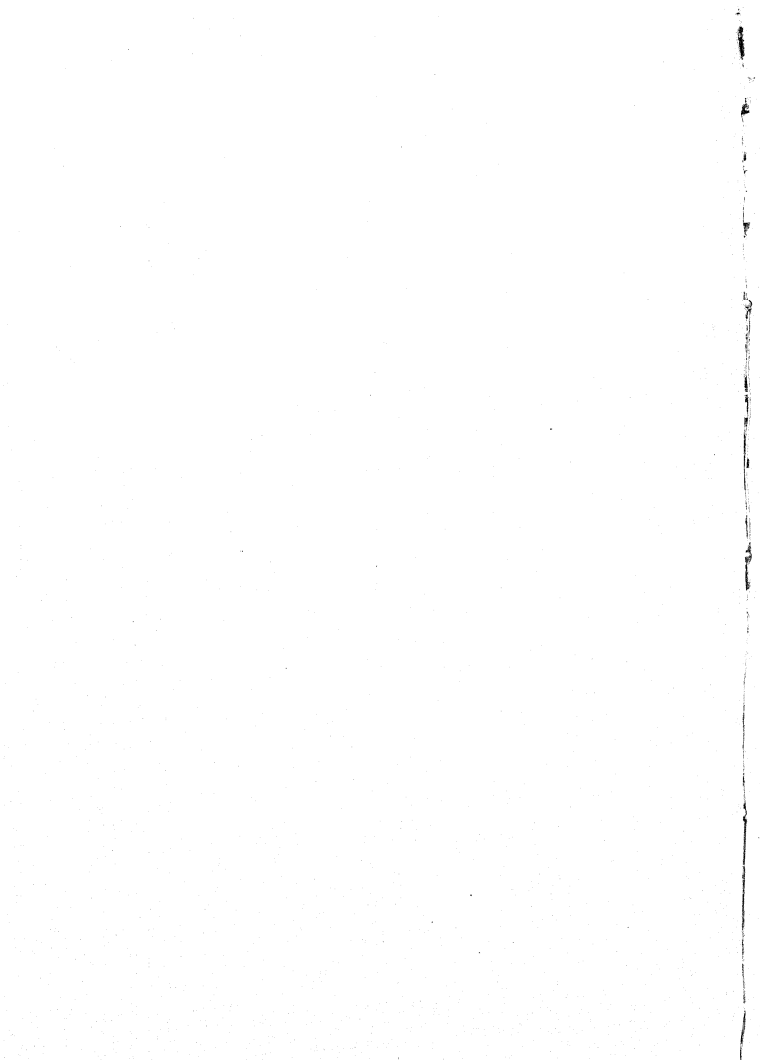
FIGS. 69-72.—*Stomiopeltella suttoniae*: fig. 69, portion of mycelium showing character of cells; fig. 70, thyriothecium showing ostiole and character of covering membrane; fig. 71, ascus with ascospores and paraphysis; fig. 72, two ascospores.

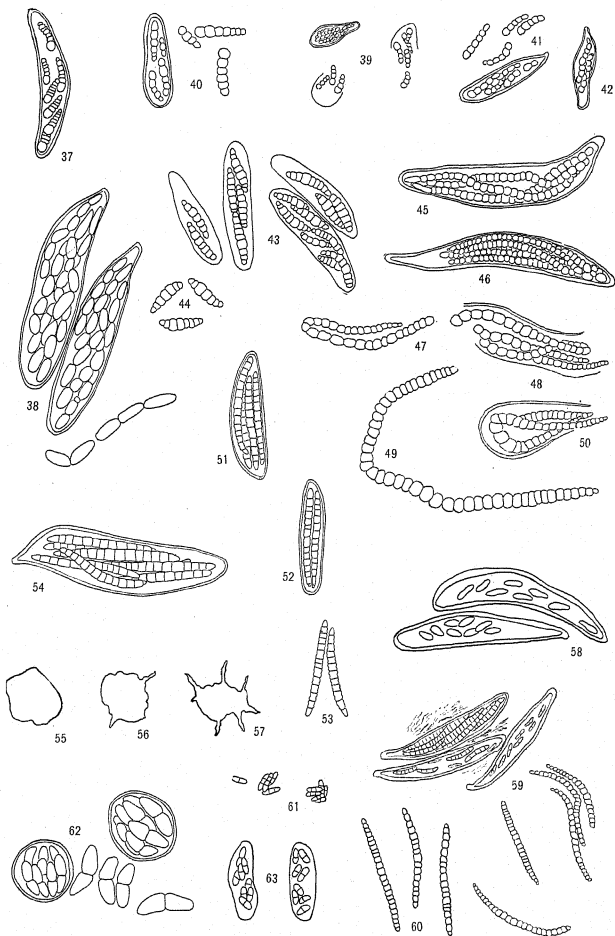
FIGS. 73-75.—*Scolecopeltidella palmarum*: fig. 73, thyriothecium showing ostiole and character of covering membrane and cells of mycelium; fig. 74, ascus with ascospores and paraphyses; fig. 75, two ascospores.

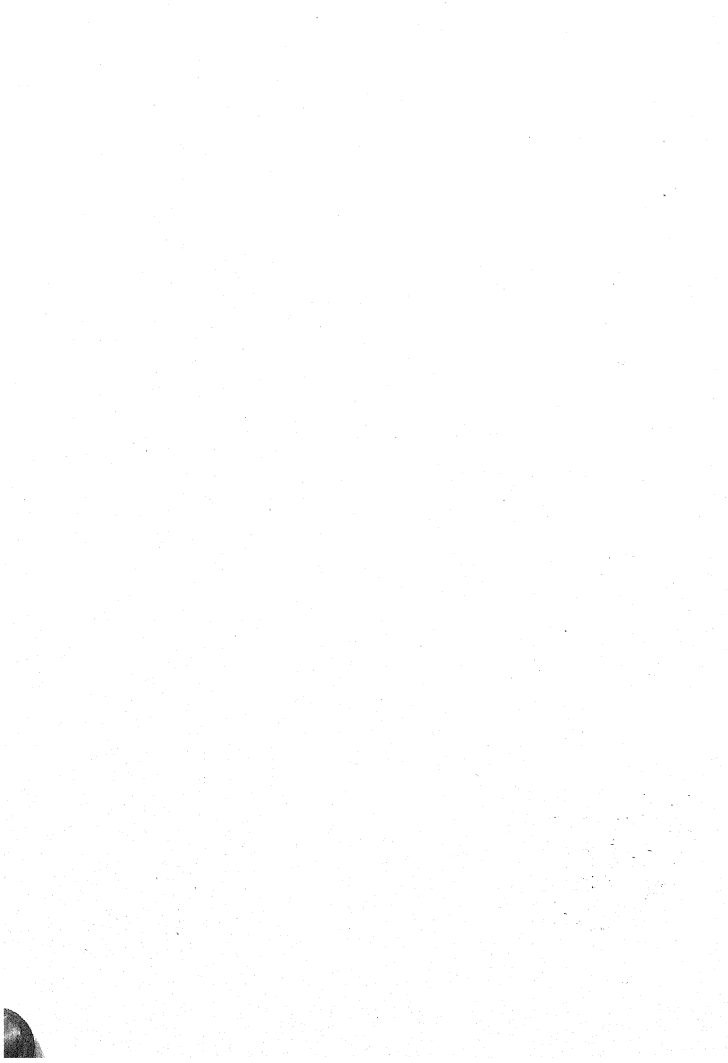


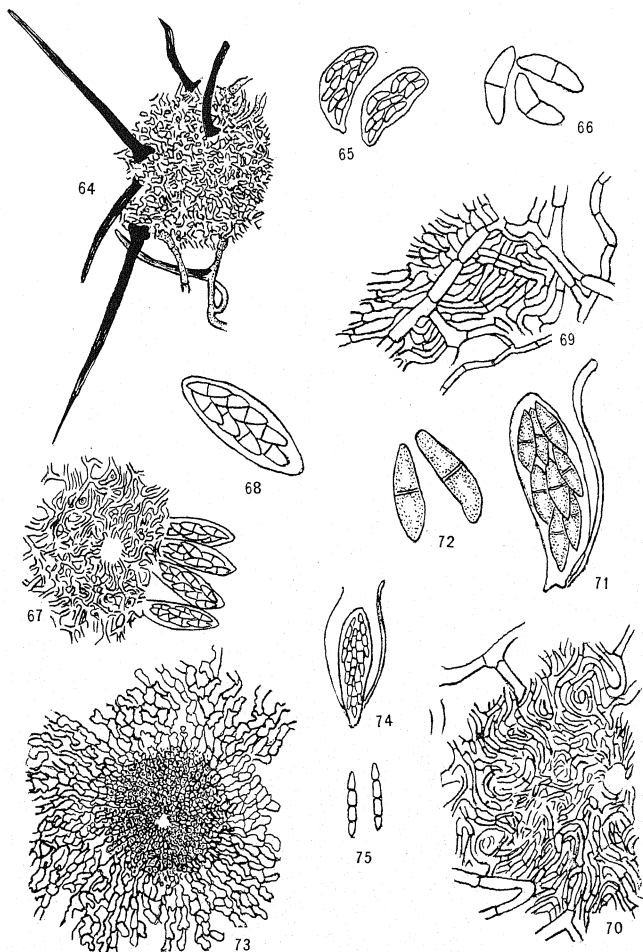


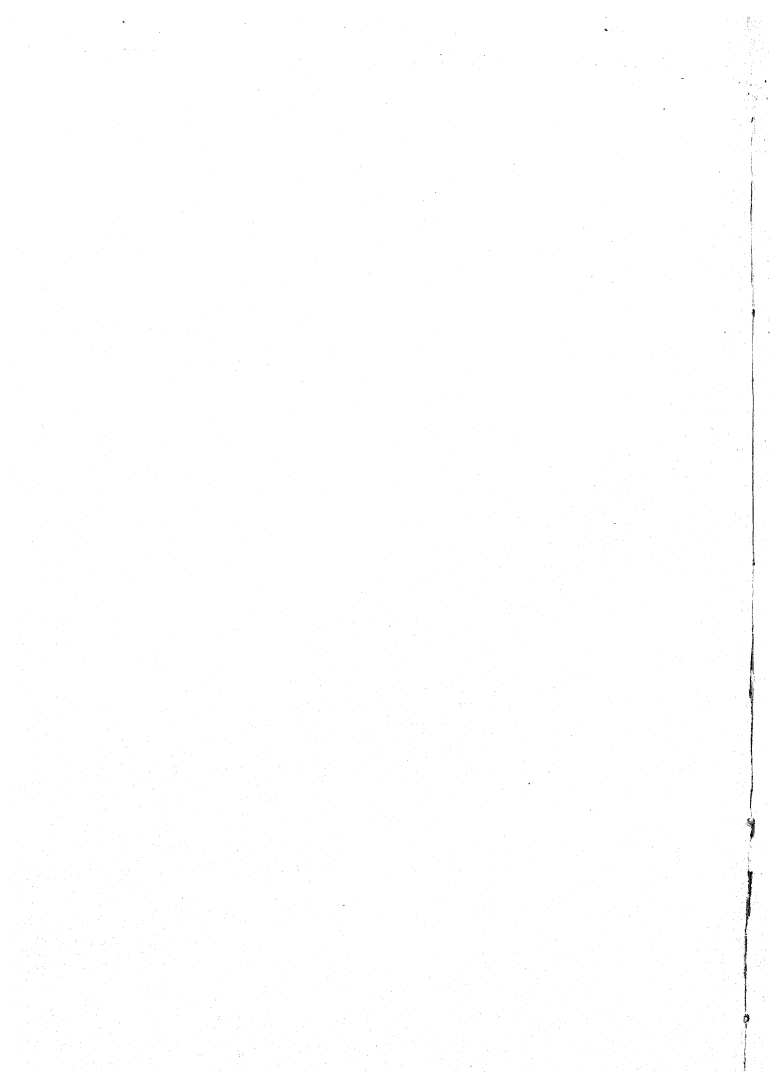












ONTOGENETIC STUDY OF PHYLLOXERA CARYAESEPTEM

A. C. MARTIN

(WITH PLATES XXII-XXV)

In the prosecution of this study the primary objective was an investigation of the developmental phases of *Phylloxera caryaeseptem* Shimer. This gall fulfills the qualifications which KUSTER (4) considered essential to "prosoplasmas," in that it possesses distinct and definite size, shape, and internal organization; tissue differentiation is not extensive but shows striking fundamental contrast to the normal leaf histology. The gall is a heteroplastic hyperplasia of the "walled" or "umwallungen" type, and in its occurrence it is restricted to the leaves of hickories. In his synoptical table to *Phylloxera* galls occurring on *Carya*, PERGANDE (5) establishes four major units, and *P. caryaeseptem* is placed with eleven other galls in group 1.

Historical

Apparently COOK (3) is the only one who has made any previous recorded histological study of *Phylloxera* galls on hickories. He examined several species, but the information which he contributed is very scant. The failure of European cecidologists to conduct research on this group is doubtless accounted for by the fact that the common hickories are restricted in range to eastern North America. So far as could be determined, the histology and morphogenesis of *P. caryaeseptem* has not been studied before.

Technique

Specimens for microscopic study were collected in the vicinity of Raleigh during April, 1922. Material was killed and fixed in the weaker solution of Flemming's fluid, or in stock chromo-acetic solution, then imbedded in paraffin, sectioned on a microtome, and stained with the safranin-light green combination. Very early stages were secured for study by close observation of a particular tree which had been badly infested with this species of gall in the pre-

ceding year. Drawings were made with the aid of a camera lucida and with a home-made projector.

Description of gall

In "Group I" the galls are "thin, paper-like, more or less transparent." PERGANDE's description of the mature gall of *P. caryaeseptem* is in part:

Their transverse diameter at the plane of the leaf ranges from 5 to 12 mm., and their vertical diameter from 4 to 6 mm. They are quite convex on both sides, and generally more conical and more prominently projecting above than beneath, especially so in the smaller specimens. All are provided with a nipple on both sides, the upper one stouter and more prominent. . . . Both nipples, particularly the lower one, lean frequently somewhat toward one side. Both openings are either round or oval and fringed by eight stout or slender filaments. The opening on the under side closes gradually, so that the insects are compelled to leave from above. The cavity, if cut vertically, appears to be more or less hexagonal at the central portion, more or less pointed toward the nipples, and quite flat at the median circumference, with the exception of a ridge which indicates the former division of the gall into two compartments while young.

The galls occur either isolated or aggregated, sometimes twenty-five or more on a single leaflet. When numerous they incline to fall below the normal size, indicating the probability that there is competition for a limited food supply. Frequently the bases of the galls are confluent, but in this event the chambers of the different galls do not merge. The characteristic location is in close relation to fairly prominent veins, but some specimens were found in which the vein relationship was not in evidence. Most of the specimens collected were a fairly bright green with or without a tinge of red near the base. The filaments on the nipples fold back conspicuously at maturity. The species is common near Raleigh, and certain local hickory trees are subject to severe and recurring annual attacks.

The temporary transverse partition to which PERGANDE makes reference, and from which the gall and gall insect derived their names, has been noted by cecidologists, but the nature and significance of this structure seem to have escaped detailed investigation. Interest in the morphogenesis of the septum served as the chief incentive for the present study.

Normal leaf

When the aphids commence activity the leaflets are about 0.065 mm. in vertical dimension, whereas at maturity the thickness is about 0.14 mm., fully twice as great (figs. 6, 7). At first the tissues are compact, but later air spaces appear, particularly in the lower mesophyll. Included within the upper and lower epidermal layers are three primitive mesophyll layers, the palisade layer and two other distinct layers below it. The central layer is relatively clear, whereas the lower one contains much granular material, and the cells of the palisade layer are spotted with chloroplasts and other matter. Perceptible vertical lengthening of the palisade cells is evident at a very early stage, and this tissue remains as a single distinct layer during the natural life of the leaf. With increasing development of air spaces in the mesophyll in advancing maturity, the two rows below the palisade layer lose their stratified identity and become organized into typical spongy parenchyma. Even before this modification in arrangement of cells, while the leaf is still very young, there tends to be some cell division in the plane of the leaf, so that the layers are not uniformly one cell in thickness. The three inner layers, however, are quite distinct at the time of gall inception.

The upper leaf surface is nearly glabrous, but the lower is thickly beset with stellate hairs and with peltate scales of two sizes (fig. 2). Exceedingly long, septate, unbranched hairs are present on the lower surface of the larger veins.

Aphid

The nymph (fig. 15) is about 0.1 mm. long and about 0.06 mm. wide. With the exception of the dark legs and the reddish eyes, it is of a bright orange-yellow color. The proboscis is very long and extends some distance behind the body. Numerous progeny of the stem mother appear in the gall shortly after the disappearance of the septum. The aphid operates on the under side of the leaf almost exclusively, and this surface is so tomentose that the insect stays aloft in the stellate trichomes during the juvenile phases of the gall, and does not come into direct contact with the leaf epidermis except as it inserts its setae. The length of the aphid's proboscis appears to be an evolutionary adjustment to the hirsute character

of the lower leaf surface, the insect's response to defensive specialization on the part of the leaf.

It might be assumed that the presence of the aphids on the lower surface, and their absence on the upper, is due to the fact that the lower surface is the first to be exposed when the folded leaf extends from the opening bud, but the correct explanation probably lies in the adaptability of certain characteristics of the lower side to the requirements of the gall maker. In the examination of prepared slides, and also in macroscopic inspection of young, living galls, instances were noted in which aphids were present on both sides of the septum of the same gall (fig. 14). These instances were exceptional and therefore do not help to explain the two-chambered character of the gall. Normally only one chamber, the lower, is inhabited, and the typical development of the whole gall is controlled by one aphid. In the cases where two aphids were found living in the same young gall, it appears that proliferation was initiated by the insect on the lower surface of the leaflet, and that the other aphid, while moving over the upper surface, chanced on the empty upper compartment and made itself at home.

Very young aphids in rudimentary galls were observed in the execution of a regular periodic contraction and extension of the abdomen. In contraction, the abdomen was reduced to about one-half its normal length and was curved downward slightly. The contraction was maintained for a second or two and then released gradually. In the particular individuals studied the movement was seen repeated at intervals of about 40 seconds. The insects escape through the upper nipple four or five weeks after the stem mother begins gall formation.

Gall development

MACROSCOPIC

The first superficial evidence of gall formation is the appearance of a small, circular, translucent area in which the aphid can be discerned. This area increases in size, and at a slight distance beyond its rim a pink or red border develops, and usually remains as a permanent character of the gall. Incident to the growth of conical walls on both sides of the leaf (figs. 1-3), the transparent area is

gradually obscured and finally hidden entirely. While the gall is still quite small, the separate chambers in it are united into one large cavity (fig. 5) by the breaking of the intervening wall. The gall increases in horizontal dimension very rapidly, and as it does so its chamber and the surface walls become extended horizontally.

MICROSCOPIC

SEPTUM.—In the early embryonic condition of the gall, the septum shows much of the typical character of the normal young leaf. Here as elsewhere there are the usual five layers, the columnar palisade cells, and the relatively clear and transparent central layer. Gradually the normal features disappear, and although the process is much less pronounced in the septum than in other parts of the embryonic gall, there is seen a tendency toward the development of a homogeneity of tissue, which WELLS (7) has interpreted as a recapitulative phenomenon characteristic of gall morphogenesis. This tendency was noticed in the palisade tissue on the loss of two normal characteristics; the chloroplasts become obscure and the typical shape disappears. In the palisade layer and the central layer the cells are larger than others in the septum, and they incline to broaden horizontally until many of them have a somewhat cubical shape (fig. 11). The epidermal layers are modified only slightly, but even in these some enlargement and horizontal elongation are evident. The tissues remain compact; no air spaces appear. All of the three mesophyll layers exhibit conspicuous hypertrophy, and the constituent cells seem to have an excessive protoplasmic content. This portion of the septum appears to be relatively quiescent meristem held in protracted dormancy.

Limited hypertrophy and horizontal elongation are not the only expressions of growth which occur in the cells of the septum. Although it is customary for this region to exhibit a minimum of hyperplasia as contrasted with the rest of the gall, some cell division does take place. This is in either one of two ways, vertically or horizontally. Vertical division, that is, division in a plane at right angles to the plane of the leaflet, occurs very sparingly in the epidermal layers, but it is not infrequent in the mesophyll, especially in the lower layer. Horizontal division in the upper epidermis of the

septum (fig. 11), causing subdivision of this layer into several rows, was noted several times and is not rare, yet it appears to be atypical, and, as will be explained below, it seems somewhat abortive. The lower epidermis, in the region of the septum, is the most static part of the entire gall; aside from very restricted hypertrophy no conspicuous growth development occurs in it. In time, all of the cells of the septum tend to flatten out laterally as the gall grows (fig. 12). The mesophyll cells carry this tendency to the extreme of attenuation, and gradually they disintegrate and collapse, leaving only the two united epidermal layers as a barrier between the upper and lower chambers (fig. 16). These changes in the septum are noteworthy, and their significance will be treated in an etiological consideration. Ultimately the epidermal layers capitulate, and the fragile barrier is broken in one or several places.

CHAMBER WALLS.—In the region bordering on the septum very active meristem develops, and here there is a rapid and pronounced approximation of homogeneity. In cross-sections of young galls, there is evident a distinct transition from the five layers of the septum to the multi-ranked condition which is attendant on active proliferation in the meristematic zone at the base of the walls. The proportionate amount of hyperplasia accruing in each of the original five layers can be determined by noticing the size and arrangement of the component cells. The mesophyll layers all divide extensively and do so approximately uniformly. The epidermal layers also divide, but generally the lower epidermis proliferates more slowly than the upper epidermis. Possibly there is some relation between this differential growth and the variance in direction of the first protrusion of the upper and lower chamber wall. The sides of the upper chamber begin to converge very early, whereas the walls of the lower chamber usually diverge at first (figs. 13, 14). In time, pronounced convergence manifests itself on both sides, and in the mature gall the walls form horizontal or gently sloping roofs, from which the chimney-like nipples project vertically (fig. 17a).

Ordinarily the walling-up process commences almost simultaneously on both sides of the leaflet, but in certain cases, already alluded to, horizontal division was found in the upper epidermis of the septum, and it was noted that there was a correlated tardiness in

the early growth of the upper cone (fig. 11). Evidence, however, indicates that even in perfectly normal galls the lower walls tend to elongate more rapidly than do the upper ones.

Practically all of the cell division in the wall region is horizontal; the cells are pushed out in vertical rows and elongate in the direction of wall development. Enormous hypertrophy appears in some parts of the gall. Fig. 14 gives the outline of a single giant cell, and included within its walls is shown normal leaf tissue in cross-section drawn to the same scale of magnification as the hypertrophied cell. The cells in all but the lowest and outermost portions of the walls are all large. Those in the nipples are much elongated. The smallest cells are found in line with the plane of the old septum, and also near the basal rim of the gall. All the cells, whether relatively large or small, are compact; no air spaces develop.

A nutritive zone develops as an inner lining of the gall chamber. This takes place after the septum has disappeared. The cells in this zone contain much dark colored material. The cell walls are thickened and appear to have gelatinized middle lamellae; no lignification is evident. Short, simple, septate hairs with swollen bases are numerous along the inner surface layer of this tissue. The entire zone consists of only a few layers of cells in the ceiling and floor of the gall, but at the sides of the chamber it covers a very extensive area. The first formation of nutritive tissue is in the cells contiguous to the septum region, and it is here that the cell walls attain a maximum of swelling. Immediately beyond the nutritive area the cells are essentially homogeneous, even though there is a wide range in size and shape. The cell walls are thin, and within the cells much stored starch is present. In the part of the gall adjoining normal leaf tissue, the part farthest removed from the gall chamber, dedifferentiation is incomplete. Vertical rows of cells containing dark material were frequent, and these rows appear to be large editions of similar ones found in the leaf. Another resemblance to leaf tissue is in the relative clearness of the cells representing the central layer.

A reticulated system of conductive vessels ramifies through the main part of the gall, and small bundles are found extending part way into the nipples. Hyperplasia seems to be particularly pronounced in veins which are included in the gall. An epidermis is

developed on the outside of the gall, but there is no distinct protective layer in the nipple region. This suggests an explanation of the premature drying and curling back of the nipple filaments. There is copious growth of unbranched, septate hairs of variable length on the inner side of the nipple. These project in the direction of the nipple mouth. The septate type of hair was noted nowhere else on the hickory leaf except on the largest veins. There is a scattering of stellate or simple hairs over the basal surfaces of the gall.

Starch grains

Very large grains, which give a very definite starch-iodine reaction, are found abundantly in many of the cells outside of the nutritive region. Some of the largest of the simple type attain a diameter of 20μ . The size of the bodies varies greatly within one gall (figs. 8, 10); the smallest are found near the basal margin of the gall and the largest are produced near the corners of the mature chamber (as seen in vertical section). There is a fairly regular gradation in size of grains in the area intermediate between these two regions. The grains in the upper half of the gall are distinctly larger, more numerous, and stain more brightly than those in the lower part. Many of the bodies are found in stages of disintegration, and throughout the gall cells and groups of cells are encountered having nothing but fragmentary skeletons of these structures. Beside the nutritive tissue and epidermis, the nipples and the lower regions of the gall are usually devoid of grains. Both simple and compound grains are found. The simple forms are spherical in shape. The compound ones are of two types. Partitions extend either a short distance or fully across the body; both conditions are obtained, so according to HABERLANDT (2) some of the starch grains are partially compound and others are completely compound. Concentric layering was obscurely shown in some specimens. The presence or absence of amyloplasts was not conclusively determined.

Three other *Phylloxera* galls on the hickory were examined for starch grains. *P. caryaecaulis* Fitch and *P. caryaeglobuli* Walsh showed numerous enlarged grains. In *P. caryaecaulis* many of the cells were literally stuffed with starch (fig. 9). On the other hand, no starch grains were distinguished in *P. caryaevenae* Fitch.

Nuclei

Appreciable enlargement of the nucleus and its nucleolus is observable in most cells of the young gall. According to measurements, both the nucleus and the nucleolus are about a third larger than normal. In cells which appeared especially vigorous, there was a more or less extensive clear area between the nucleus and the nucleolus; in contrast, other cells showed constriction of the nuclear membrane, dim nucleoli, and much granular matter in and about the nucleus. The latter symptoms were found often in cells of the lower epidermis of the septum, and also appeared in many aging cells in other parts of the gall. In the cells of the juvenile stages the nuclei are suspended near the center of the protoplast. HORSFALL (3) calls attention to a tendency of nuclei to orient themselves within the cell toward the side near a puncture path made by the insect. Particular examination on this point in *P. caryaeseptem* gave no confirmation of HORSFALL's observation. The only recorded tendency toward a particular orientation was in the upper epidermis of the septum. Here the nuclei are frequently located on the lower walls of the cells. Normal mitotic figures were found in a number of nuclei.

Aphid punctures

The cells in and adjacent to a wound normally suffer partial collapse. This tendency facilitates the detection of the course taken by the piercing mouth parts of the insect, and makes it possible to discover the location of different wounds. Apparently the very first puncture is indistinguishable from others; its location was not determined. Very early insertions of the setae were noticed in the tissues which give rise to the chamber walls, and it may be that the filaments which constitute the terminus of the nipple have their origin in definite relation to these punctures. Almost invariably punctures were made in the tissues just beyond the point of origin of the walls. Very frequently large veins bound part of the gall, and in such cases puncture channels appear in the vein, and also in the tissues immediately beyond. In that part of the gall circumference where a vein is not present, a slight contraction of the leaf thickness was perceived near marginal punctures. Most of the early wounds made

by the insect traverse the entire thickness of the leaf. Possibly this fact is related to the double chambered character of the gall.

The paths of the setae are partly intracellular, but are mostly intercellular. They appear to follow the course of least resistance through the middle lamellae, and take short cuts through the cells only occasionally. Here, as in HORSFALL'S studies, side branching from the original channel was found. This seemed to indicate that the insect had partly withdrawn its setae and had then plunged them into fresh tissue. Enveloping sheaths which developed around the setae, and which extend outward a variable distance from the end of the channel, were common. A few instances were noted in which a sheath was formed on the upper side of the leaflet, on the opposite end of the channel from that at which the puncture was made. While the septum is intact it serves as the favorite feeding location of the aphid. Even when nothing but the epidermal layers endures as evidences of the original leaf tissue in the septum, the insect continues to suck through the remaining thin structure. After the septum breaks, the aphid still works on the rim of the septum or probes into closely adjoining tissue.

In regard to response from setal insertion, and also in regard to number of punctures, my observations conflict with those of ROSEN (6), who made a study of the development of the gall built by *P. vastatrix* (*P. vitaeifoliae*). ROSEN conducted experiments in which he produced galls artificially by the use of chemicals. He states:

It will be seen that in the intumescences produced by the application of chemicals, the place of application is the place of excessive growth, and in the *Phylloxera* the place of application is the place of hindrance of growth. From these experiments, the burden of proof becomes more difficult for those who adopt the "chemical" theory of gall production for sucking insects.

In the present study of *P. caryaeseptem*, the setal paths were found not only in the parts where growth is slow or lacking, but they also occur conspicuously in the regions of rapid proliferation. Even disregarding this fact, it seems to the writer no more difficult to explain the inhibition of growth in the locality of application of an "initial" stimulus on a chemical basis, than on a mechanical or physical one. In the case of *P. vastatrix*, ROSEN concluded that the insect made but one leaf puncture, and that in this puncture the setae remained

more or less permanently fixed. Similar habits have been noted in certain other gall makers, by other investigators. In *P. caryaeseptem*, however, the punctures are numerous. In speaking of the apparent immobility of the insect in the gall he studied, ROSEN states that "A proof of this (immobility of the insect) is the frequency with which I have found the proboscis in the tissues in many of my prepared slides." The same tendency for the setae to break off and remain in the puncture was observed very often in *P. caryaeseptem*, but in this gall it was clearly evident that loss of the setae did not indicate immobility of the aphid.

Etiology

Although it appears to the writer that tension, and such physical changes as are wrought by the sucking of the aphid, should not be considered as the primary factors in gall formation, still these phenomena may play a conspicuous rôle in determining certain characteristics of the gall, and therefore they are mentioned in an etiological connection in this paper. Evidence which indicates that there is exertion of tension on the septum is deduced from certain features of gall development. Cells of the septum which at first have their long axis in a vertical direction, later become elongated horizontally. Furthermore, measurements show that from the period of gall incipency until the stage when the septum breaks, the horizontal diameter of the gall cavity is either doubled or trebled. By the time the gall is nearly mature, the diameter has increased 5-12 times. It seems logical to assume that this growth in width of the gall cavity tends to stretch the septum horizontally. While it seems obvious that tension is a factor in transforming the septum, however, it might be maintained that the sucking of the insect acts concurrently with tension, and wields considerable control over the alterations which take place in this part of the gall. Support for this inference might be cited in the collapse and disappearance of the mesophyll cells previous to the disorganization of the epidermal tissues. If growth tension is considered the only influence active in changing this part of the gall, it may appear difficult to reconcile the sole mechanism of this force with the premature disappearance of the mesophyll layers. For those who discount the concept which

ascribes etiological importance to the bodily removal of material from the leaf by the aphid, the logical recourse seems to lie in the search for peculiarities of the epidermis, which would account for the comparative tenacity of this tissue. Mere thickness and strength of cell walls do not explain why the epidermis persists while the mesophyll cells elongate and collapse. There is one feature of the epidermis, however, which tends to render the tension theory self-sufficient and make the suction explanation superfluous. It is the fact that vertical cell division occurs in the epidermis while the septum is being stretched. In vertical sections through young galls 12-25 epidermal cells were counted, whereas in more mature septa the number ranged between 30 and 45. The mesophyll achieves temporary accommodation to the lateral strain through the elasticity of its cells. Possibly the endurance of the epidermal layers signifies that in this case adjustment to continued strain is more efficiently coped with by cell division than by cell plasticity. It seems certain that growth tension is a factor in modifying the septum, but the rôle played by aphid suction appears dubious.

Summary

1. Two conical "up-walled" chambers, produced by one aphid, develop concurrently on opposite sides of the leaflet, and a partition consisting of modified leaf tissue is left as a septum between the compartments.
2. Within the septum the tissues approximate a homogeneous condition, and remain as relatively inactive meristem, in which some hypertrophy is manifest, but in which only a very little hyperplasia occurs.
3. The septum is an evanescent structure, and in its deterioration the mesophyll cells attenuate horizontally and disappear, bringing the two epidermal layers into a temporary union. Eventually the epidermal layers break and the two gall cavities become one.
4. Growth tension appears to be the main factor in transforming the septum.
5. In the region bordering on the septum, the meristem, dedifferentiated from the original layers, expresses itself in prolific activity. Reversion to the primitive meristem condition is rapid, and is

accompanied by extensive hyperplasia. Very pronounced hypertrophy ensues in most of the cells.

6. In point of diversity this gall displays limited tissue differentiation. A nutritive zone, an extensive parenchymatous region containing vascular tissue, and an epidermal layer covering certain parts of the gall's surface are the chief tissues.

7. Setal punctures are numerous within one gall. Sheaths are often associated with the punctures.

8. Puncture channels were found frequently in tissues of active proliferation, as well as in regions of retarded growth.

9. Greatly enlarged starch grains develop in some cells of the parenchyma.

The writer is indebted to Dr. B. W. WELLS, of the Botany Department of North Carolina State College, for information and much considerate assistance in this study.

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DESCRIPTION OF PLATES XXII-XXV

PLATE XXII

FIG. 1.—Upper cone of very young gall.

FIG. 2.—Lower cone of very young gall.

FIG. 3.—Vertical section of gall shown in figs. 1 and 2, showing septum between two developing chambers.

FIG. 4.—Gall which is nearly mature.

FIG. 5.—Vertical section of gall shown in fig. 4; septum rim present in center.

FIG. 6.—Vertical section through normal young leaflet, showing characteristic tissue arrangement in five rows.

FIG. 7.—Vertical section through more mature leaflet, drawn within walls of greatly hypertrophied cell; both leaf tissue and hypertrophied cell drawn to same scale of magnification.

FIG. 8.—Enlarged starch grains in cells of gall parenchyma.

FIG. 9.—Cells of *Phylloxera caryaecaulis*, showing many enlarged starch grains.

PLATE XXIII

FIG. 10.—Extremely large starch grains in different region of same gall used in fig. 8.

FIG. 11.—Very young gall in vertical section, in which was abnormal horizontal division in upper epidermis of septum; in this stage mesophyll cells of septum are seen to be rather cubical.

FIG. 12.—Septum of fairly young gall, showing horizontal elongation of cells.

FIG. 12a.—Outline of vertical section of fairly young gall; shaded portion represented in detail in fig. 12.

FIG. 13.—Vertical section of normal young gall.

FIG. 14.—Unusual type of gall, in which aphid is present in each chamber.

FIG. 15.—Immature stem mother.

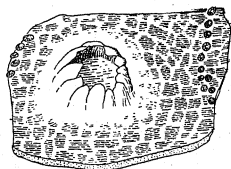
PLATE XXIV

FIG. 16.—Mesophyll cells of septum showing collapse, bringing the two epidermal layers into contact.

PLATE XXV

FIG. 17.—Vertical section through part of cavity and walls of mature gall; nutritive zone and remnant of septum indicated.

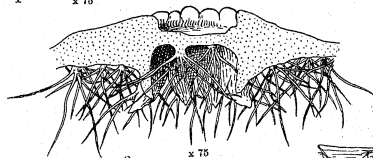
FIG. 17a.—Shaded portion of this gall drawn in fig. 17; upper nipple of gall does not show because section is not perfectly median.



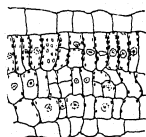
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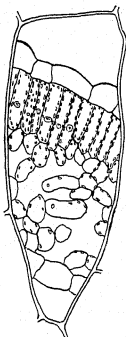
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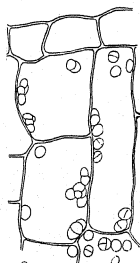
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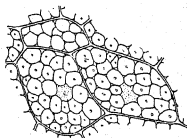
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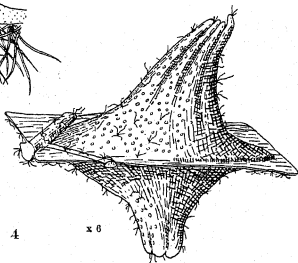
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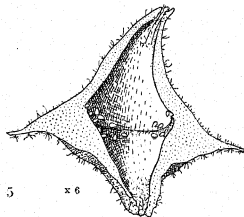
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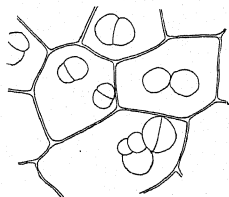
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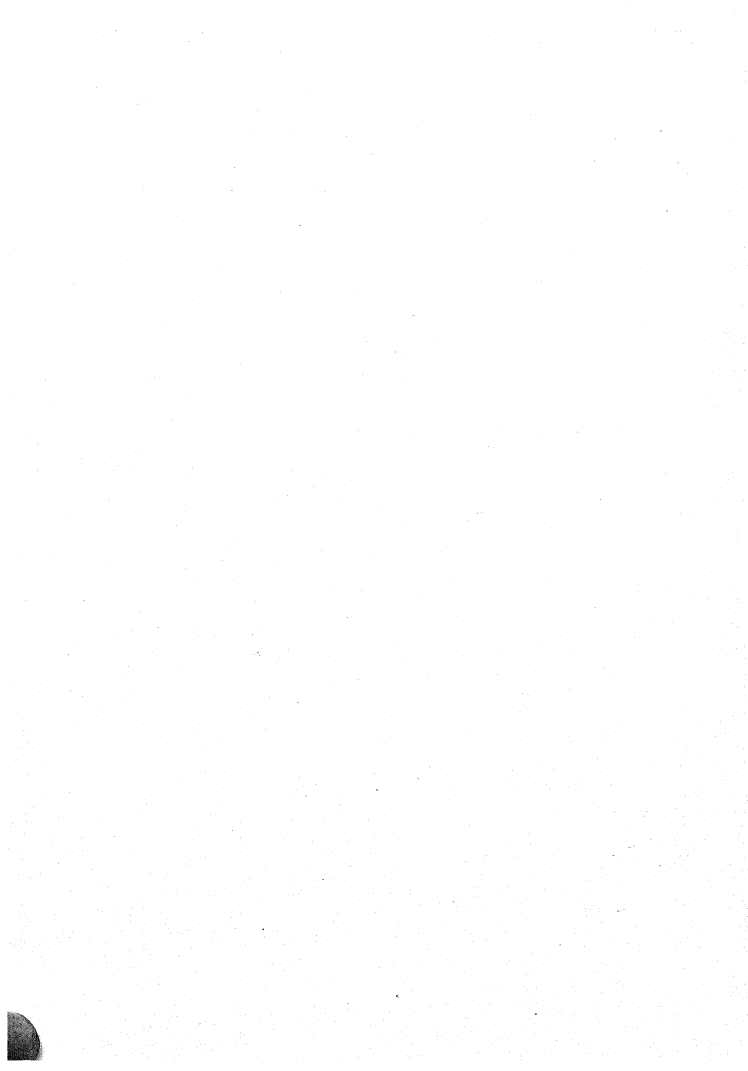
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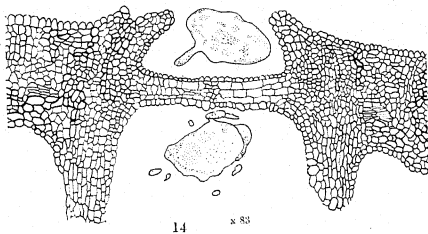
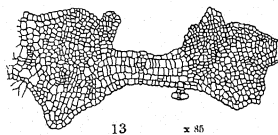
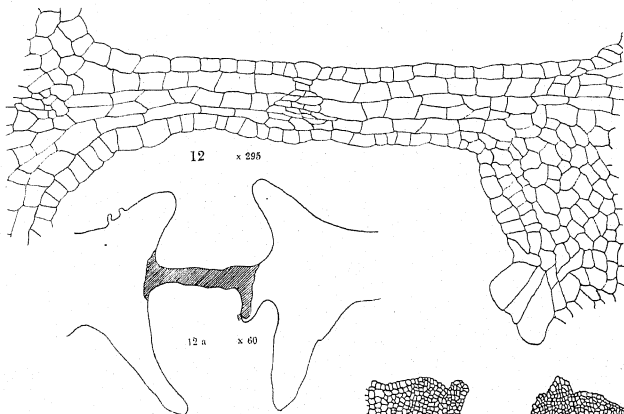
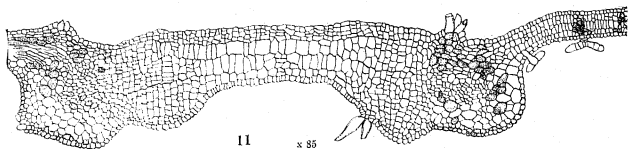


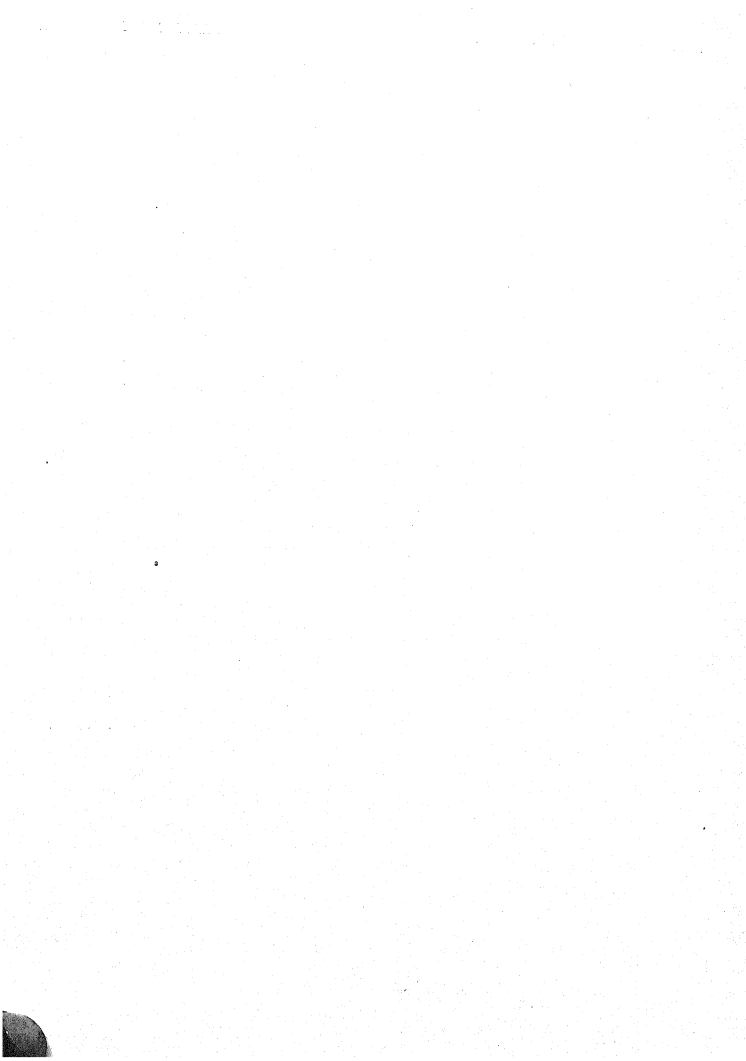
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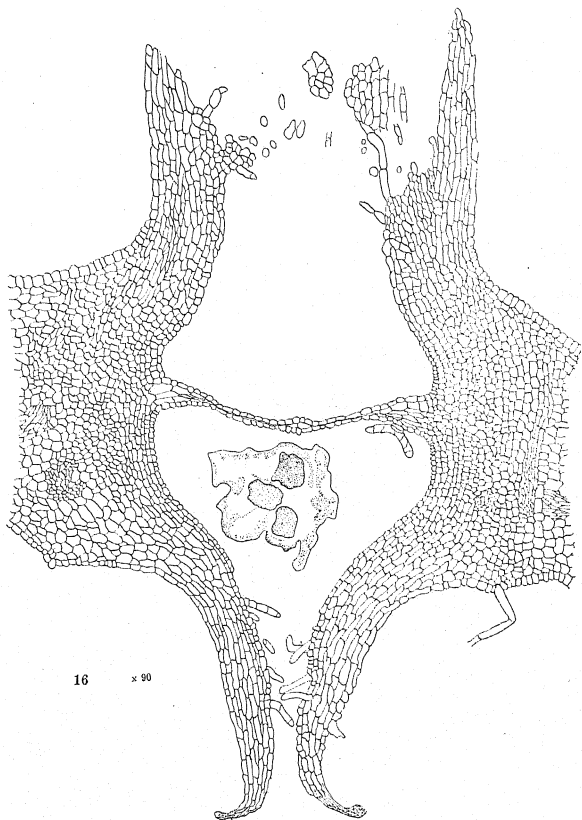


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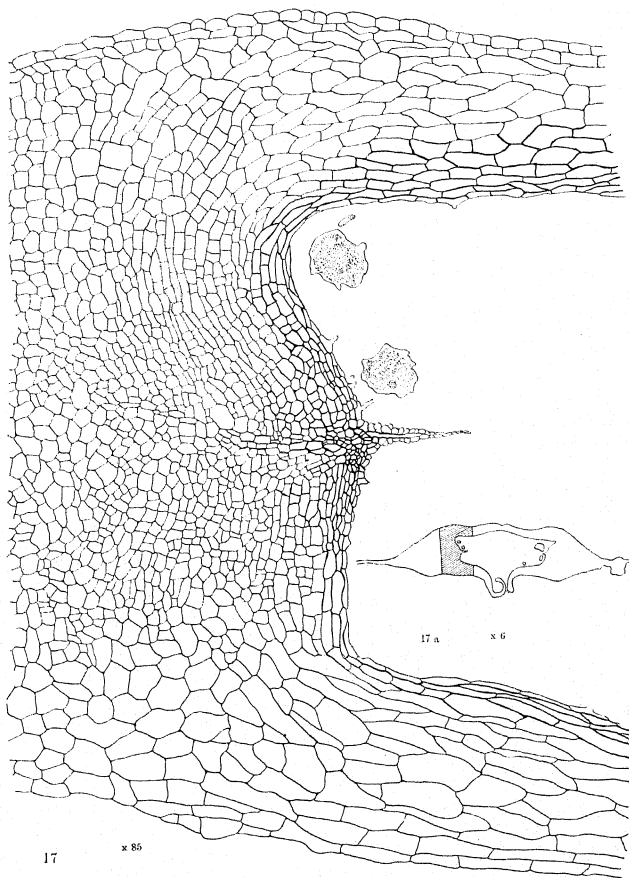




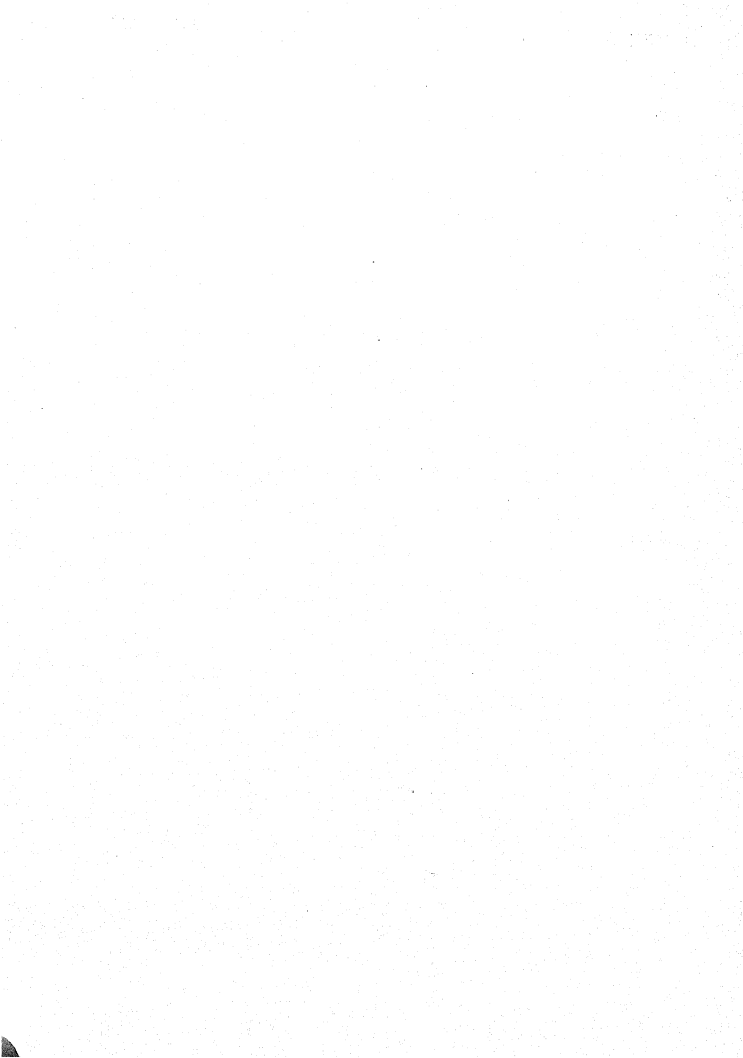
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MARTIN on PHYLLOXERA





MARTIN on PHYLLOXERA



GROWTH STUDIES IN FOREST TREES^{*}

III. EXPERIMENTS WITH THE DENDROGRAPH ON *FRAXINUS AMERICANA*

J. ELTON LODIEWICK

(WITH TWO FIGURES)

Two previous papers in this series have been published by BROWN (2, 3). Upon his suggestion the writer is continuing investigations along similar lines, and has adopted the same title for further contributions to the knowledge of growth in trees.

Very little has been published as to the season of cambial activity in the broad leaved deciduous trees of the northeast, although the experiments of MACDOUGAL and his co-workers in the west and southwest have yielded some interesting results, and show promise of many more. In order to render these most useful, however, they should be checked by histological studies carried on simultaneously, but to date no such work has been published, and interpretations of the growth curves obtained have been based wholly on dendrograph records, unsupported by microscopical data. During the season of 1923 such studies were undertaken by the writer, to determine the accuracy of the growth curves obtained with the dendrograph, and to further our knowledge of the value of such records in computing xylem increment. In addition, a growth intensity curve showing a few points of interest has been plotted.

The dendrograph, one of the latest models, was procured from D. T. MACDOUGAL by the New York State College of Forestry. The structure and operation of this instrument have been fully described by the maker (9), but a brief summary may be helpful. It may be considered as composed of three portions: a basal belt of blocks, a floating frame, and a recording device (fig. 1). The first is a series of wooden blocks so hinged together at the ends that the whole may be placed around a tree and drawn tight by means of two

^{*} Contribution from the Department of Wood Technology, New York State College of Forestry, Syracuse, N.Y.

thumbscrews. This belt acts as a support, and serves to hold the recording instruments in the same relative position to the tree under all conditions. The second portion is a polygonal frame of narrow invar bars, which are slotted at the ends and clamped solidly together by special bolts. This may be composed of any even number of bars above four, depending upon the diameter of the object measured; six were necessary to encircle the tree used in the present in-

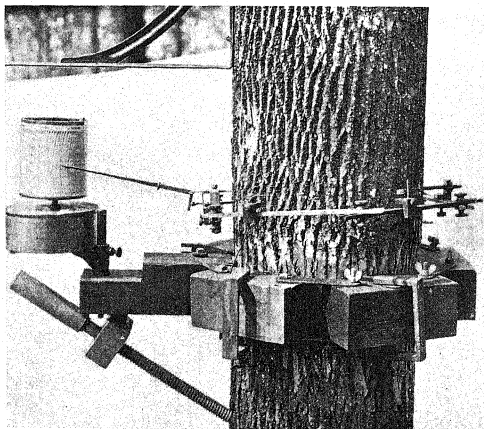


FIG. 1.—The dendrograph

vestigation. This floating frame, so-called because it is not directly in contact with the tree at any point, is supported about three inches above the basal belt on four slender wires. At the center of one bar is an adjustable contact screw, blunt at the apex, which is in contact with the tree, and fastened to the bar at the base. Diametrically opposite to this the frame bears a movable quartz rod, in contact with the tree at one end and operating against the short arm of a pen-bearing lever at the other. The rod is so arranged that it can

be brought to bear at any of three points along the short lever arm, thus giving three possible magnifications of the diameter fluctuations of the tree. The third and recording portion of the instrument consists of an eight-day clock operating a cylinder covered with a ruled record paper, and attached to a large block in the supporting belt.

The tree selected was a forest-grown specimen of *Fraxinus americana* L., growing in a sandy loam deficient in humus, and on an east slope at the College Experiment Station about three miles south of Syracuse, New York. This individual was approximately fifty years of age, thirteen inches in diameter, about forty feet tall, and free of branches for eighteen feet. The crown was well lighted from above, but one-sided because of shading on the north and east. The dendrograph was attached at breast height to measure the north-south axis, and adjusted to magnify diameter changes 12.5 times.

Probable errors in dendrograph

Four apparent opportunities for error are present in the dendrograph because of its structure and method of operation. The first, the variation resulting from the coefficient of expansion of the materials used, has been shown by MACDOUGAL to be negligible. Computations of the possible variation under normal daily temperature ranges show it to be too slight to be recorded, even under the highest magnifications obtainable.

All growth taking place under the movable quartz contact is registered directly, but the diameter increase on the opposite side of the trunk can be recorded only by a movement of the whole floating frame toward that side of the tree. To move the frame thus, the supporting wires must be bent, hence some pressure is exerted under the stationary contact screw in order to overcome this inertia. It was thought possible that some atrophy might occur under the contact screw as a result. On October 8 the dendrograph was removed and a series of cuttings made at this point, controls being taken at distances of one inch on all four sides. Careful measurements of the ring width with the microscope failed to show a narrowing of the xylem increment because of applied pressure, eliminating this factor as a probable source of error.

During the season the basal belt of blocks was not loosened; hence some pressure and consequent atrophy might have occurred under them. If the constriction were great enough to retard food transportation, the ring should be narrower under and below the blocks and wider above, and the increased growth might be transmitted far enough to be recorded by the contacts. Three series of cuttings were made when the instrument was removed in October for these tests. Each series began below a basal block and ended about two inches above the floating frame, with cuttings taken at intervals of about an inch. In none of the three series, taken under different blocks, was a change noticeable in the ring width; hence the dendrograph apparently does not interfere with normal food transportation and causes no modification of the ring width.

Because of the heavy furrowed bark of *Fraxinus americana*, it was necessary to cut away small areas of the periderm to provide smooth bearing surfaces for the contact rods, as well as to reduce to a minimum any hygroscopic changes in this layer caused by atmospheric moisture. The white living inner bark of the tree was approximately 5 mm. thick, and the contact points were placed directly on the outer portion of this layer. Drying of the exposed tissues was to be expected and did occur during the first week. Later a new layer of periderm formed, and cuttings from these areas in the autumn indicated that drying and consequent checking had occurred to a depth of about 2 mm., but was never deep enough to interfere with cambial activity. This justifies the assumption of MACDOUGAL that excessive drying would not follow exposure of the moist inner phloem. The newly formed periderm was not thick enough to affect materially the growth curve.

Progress of growth

The dendrograph was set up on April 11, but the record was of no value until a week later because of adjustments of the instrument necessitated by the drying of the exposed living tissues. After April 18 compensating variations were evident, and the logical supposition is that these were in progress previously.

The daily variations observed by other workers in all of the species investigated were present. On clear days, especially those which

were warm and windy, the curve showed a decided drop between 8 A.M. and noon, which continued until about 6 P.M., then rose slowly to a maximum after midnight, thereafter remaining constant until the drop of the following noon. MACDOUGAL has assumed this noon depression to be an indication of decreased turgor, and to be due to the rapid transpiration of midday. A water depletion in the stem develops, since the roots absorb water at a uniform rate and fail to maintain the usual water balance. If this assumption is correct, the noon depression should be at a maximum when the tree is in full leaf, and it follows that the greater the capacity of the air to absorb water, the greater should be the depression, all other factors being equal. The average drop of midday computed from the data for the season of 1923, from April 18 until the day that the buds broke open (May 6) was 0.54 mm. Between the latter date and September 22, the time of complete defoliation, the average depression was 1.64 mm., or roughly three times as great. From September 22 until the end of November the daily average was only 0.19 mm. No reason for the discrepancy between the depression before the growing season and that after defoliation can be advanced, other than that an appreciable evaporation takes place from the swelling buds. The depression during the period of leaf unfolding tends to average slightly higher than that occurring when the leaves are completely expanded. Whether or not this is dependent upon the structural development of the leaves and twigs must be reserved for future investigations.

The evaporating power of the air is dependent mainly upon temperature, humidity, air movements, and sunshine. An examination of these data for the season shows some correlation between the amount of noon depression and the power of the air to absorb water, but no definite figures can be given because of the inability to assign a constant to each factor. Further investigations covering these factors are planned for the ensuing season.

With only one growth intensity curve covering a single season it is inadvisable to draw conclusions as to the effect of external conditions upon the growth rate. Attempts were made to correlate daily changes with meteorological data, but without results. Several investigators, especially FRIEDRICH (5) and KORSTIAN (8), have in-

licated that a decided drop in temperature is followed by a decrease in the growth rate. This must act as a shock, because in the cases observed the temperature did not reach a point sufficiently low to inhibit growth. On June 5 and June 27 respectively, a decrease in temperature great enough to cause a decided decrease in the growth rate was observed. On the two succeeding days in each case the growth intensity again increased, but the temperature continued to fall. Before and after the period of diameter enlargement the compensating variations appeared to be closely related to precipitation, as shown by the growth-intensity curve (fig. 2). An appreciable precipitation is followed shortly by an increase in diameter, which is lost following the rainy period. This is not to be considered as growth, since no permanent increase in size results. On the other hand, such a phenomenon might be caused by hydration of the bark layers during times of high humidity, but the irrigation experiments of MACDOUGAL eliminate this factor, yet give quick increases in diameter.

Histological studies

Histological studies, aiming to correlate the dendrograph record with the progress of growth within the tree, are probably the most needed investigations at the present time. Cuttings were made at intervals of about two weeks in a manner similar to that used by BROWN (2). Small pieces showing phloem, cambium, and xylem were removed from near the height of the floating frame and close to the bearing points. Two objections to this method are possible, namely, the effect of removal of pieces on reduction of the internal pressure, and the effect of callus formation on the size of the annual ring in subsequent cuttings. Any decrease in tension through cutting should be evident in the dendrograph record, but this was not observed. The second objection was circumvented by making all the cuttings outside the range of callus formation. In addition, no incisions were made sufficiently close to the bearing points that the added growth resulting from callus formation would be effective in these areas.

The pieces removed from the tree could not be sectioned without imbedding because of the thin walled cambial layer, hence the tissues were killed and fixed in chromo-acetic fluid, softened in hydrofluoric

acid, imbedded in celloidin, and studied with the aid of both temporary and permanent stains.

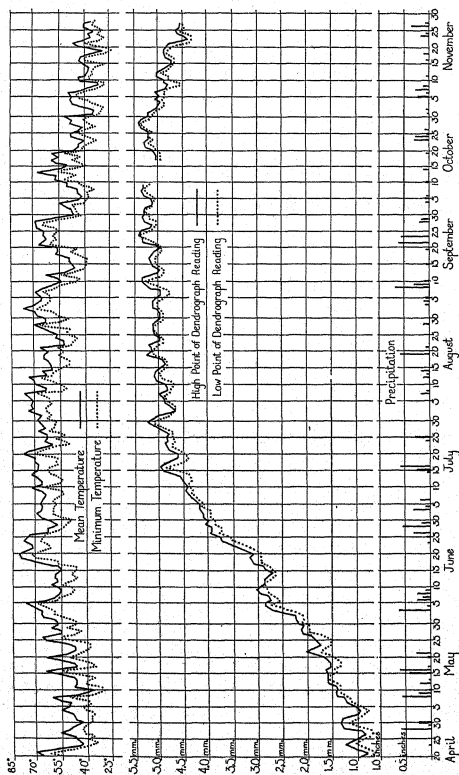


FIG. 2.—Growth intensity curve during 1923, as plotted from dendrograph records

The buds on the ash were swelling noticeably on April 20, and had broken open on May 6; the leaflets were unfolding on May 15, and were one-fourth to one-third full size on May 30. The tree may be considered as in full leaf in mid-June. The first cutting, taken May 9, showed that the first row of springwood vessels had been formed, but the vessel walls were still thin and unligified. On May 30 the second row of vessels was developing, but as yet only the primary wall was visible. The spring wood zone was complete on June 13, so far as number of cells was concerned, and nearly half of this was still unligified. On July 11 the last row of summer wood ducts had just arisen from the cambium; while a subsequent cutting on August 9 showed a ring complete so far as cell number was concerned, but with the major part of the summer wood fibers still unligified. Later cuttings showed no change in the annual ring, except for wall thickening and accompanying lignification.

The beginning of phloem formation appears to vary in different species of trees. STRASBURGER (12) indicated that in general xylem elements formed some time before the phloem elements in the spring, and that the phloem continued to develop until the end of the vegetative season. PFEFFER (11) reached the same conclusions. BROWN (3) concluded that in *Pinus Strobus* phloem and xylem formation begin simultaneously, and that phloem continues to be formed until the end of the growth period.

In *Fraxinus americana* the end of seasonal activity is marked in the phloem by two rows of radially compressed cells. The measurements of phloem widths at various times during the growing season are recorded in table I. It is evident that the first phloem elements of the season are formed several weeks after the first xylem cells are differentiated. It will be shown subsequently that it is reasonable to conclude that phloem development continues at least until defoliation in the autumn.

The first point at which the curve of growth intensity portrayed an increase above the higher points of previous compensating variations was on May 8, and cuttings taken on the following day indicated an increase in xylem, as previously stated. It does not seem probable that all this growth could occur in one day, and it is logical to conclude that cambial activity started at breast height in the bole

some time between April 29, the time of the last high point dependent on swelling of the tissues, and the date on which growth was actually recorded. If this is true, cell division must have taken place when the tissues were not fully distended. As has been stated, the buds were breaking about May 6, hence cambial activity in the base of the trunk began simultaneously with this phenomenon. After growth had once started, it continued at a fairly even rate until June 9; then a rest period of approximately ten days was evident, accompanied by shrinkage in diameter. It is to be noted that this was also a period of decreased temperatures and no precipitation. If either of these factors is to be considered as causing the rest

TABLE I

COMPARISON OF DIAMETER INCREASES REGISTERED BY DENDROGRAPH AND MEASUREMENTS OBTAINED FROM CUTTINGS

Date	Width of xylem (mm.)	Width of phloem (mm.)	Width of cambium (mm.)	Total diameter increase (mm.)	Total diameter increase according to record (mm.)	Percentage growth shown by cuttings as recorded
May 9.....	0.351	0.078	0.000	0.854	0.100	11.7
May 30.....	0.577	0.078	0.202	1.714	0.850	49.6
June 13.....	0.858	0.062	0.310	2.460	1.700	65.0
July 11.....	1.840	0.062	0.429	4.662	3.150	67.5
August 10....	2.292	0.062	0.449	5.606	3.900	69.5
October 8....	2.341	0.062	0.468	5.742	4.050	70.7

period, it would of necessity be the temperature, since the resumption of cambial activity was coincident with the approach of higher temperatures, but preceded precipitation by nearly a week. Moreover, several days of precipitation occurred just before the cessation of cambial activity, so that the soil undoubtedly contained plenty of water for growth phenomena. FRIEDRICH (5) and JOST (7) have noted a similar resting period in hardwoods and conifers, but their methods are open to criticism. MISCHKE (30) observed the same phenomenon in *Pinus silvestris* and *Picea excelsa* during the latter part of June, and attributed it to the low precipitation of preceding months. BROWN (3) found a comparable cessation of cambial division in *Pinus Strobus* at the same time of year, and concluded that the first period of cambial activity is at the expense of stored food reserves, while the second results from food manufactured in the

new leaves. Such an explanation seems more plausible in white ash, since the tree was not in full leaf until mid-June, which date just preceded the beginning of the second growth period. It is worthy of note that the rest period occurred between the formation of spring wood and summer wood elements.

Growth intensity was high during the first part of the second growing period, but gradually decreased toward its close. A period of rest is evident between July 16 and 27, for which no reason can be assigned. Few xylem elements were formed later than August 1, after which date only equalizing variations were present. On September 19, however, a new maximum was established, 0.1 mm. higher than that of July 30, and this was further increased on September 24 by 0.05 mm. It should not be inferred, however, that these new maxima were the result of xylem formation. Previously mentioned investigators, especially BROWN (3), have noted the continued formation of phloem elements in various species until inhibited by the advent of low temperatures incident to autumn, and early winter. The phloem in *Fraxinus americana* is so irregular that no definite counts of phloem elements could be made to determine whether or not such an explanation would account for the new maxima in this species. Examination of the cuttings taken during this period indicated that such a conclusion was plausible. The break in the curve in October resulted from readjusting the instrument upon the tree, following its removal to obtain the test cuttings already described.

After the end of October a more or less steady decrease in diameter was registered. This may have been caused in part by the release of pressure, and in part by drying occasioned by the large number of cuttings which had just been made. On the other hand, MACDOUGAL has observed such a contraction in the trunk in all species examined by him; hence a decrease may normally be expected at this time, although the amount is probably influenced by the factors mentioned.

Interpretation of growth records in terms of xylem increment

The ring width on different dates was determined from the cuttings made near the stationary contact screw on the south side of the tree. Xylem, cambium, and phloem widths were computed, and doubled to give the total diameter increase, with the results given

in table I. From these data there is no apparent agreement between the diameter increment as recorded and as measured. This may be explained in part by swelling consequent to the preparation of permanent mounts. FRENCH (4), in attempting to determine the specific gravity of wood by computing the actual volume of cell walls on cross-sections, found that his results were consistently high. He concluded that swelling to the extent of twenty-five per cent had taken place during the processes involved in the preparation of his slides. BROWN (1) determined that in wood of *Tecoma* treated with hydrofluoric acid, an average increase of 29 per cent resulted in the radial direction, and was accompanied by a longitudinal decrease of over 20 per cent, and a tangential increase of over 40 per cent. If similar changes took place in *Fraxinus americana*, the measurements of column 4 in the table should be reduced about 30 per cent, and would then correspond more closely with those of the dendrograph record.

The earlier cuttings of the year show a greater discrepancy, the dendrograph recording but 12 to 15 per cent of the growth as computed from permanent mounts. As stated, a portion of this might be attributed to a swelling of the tissues resulting from desilicification. The remainder is possibly due to an irregular awakening of the cambium. In general, cambial activity on the south side of trees precedes that on the north, because of better insolation; but growth, once actuated, progresses more rapidly on the north side. Near the end of the growing season the ring width is comparable at the two points. All measurements in this investigation were made on the south side, and it is possible that growth was more tardy on the north side; hence the discrepancy obtained by doubling the measurements. Further investigations are necessary, and these explanations of differences between the dendrograph record and actual measurements are advanced only as possibilities, and not as conclusions drawn from actual data at hand.

Summary

1. Growth studies were made during the season of 1923 on *Fraxinus americana* L. by means of a dendrograph. Correlated histological investigations were made on the same tree.

2. No distortion of ring width is caused by the contact points or basal blocks of the dendrograph.

3. The desiccation of layers of phloem exposed in setting the instrument does not interfere with cambial activity.

4. The noon depression in diameter is greatest when the tree is in full leaf, supporting the supposition that this is brought about by the water relations of the tree.

5. A decided drop in temperature is usually followed by a decrease in or a cessation of growth.

6. The compensating variations before and after the growing period are closely correlated with precipitation.

7. The first xylem elements at the base of the trunk were formed simultaneously with the breaking of the leaf buds. Xylem formation ceased in this region about August 1.

8. Phloem formation began about three weeks after the first wood elements were formed, and continued at least until the defoliation of the tree on September 22.

9. A rest period of ten days occurred in early June which could not be correlated with external factors.

10. The dendrograph registers less growth than is shown by cuttings, but no definite explanation can be given for this phenomenon.

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FORMS OF NITROGEN IN PURE CULTURES OF ALGAE¹

E. B. FRED AND W. H. PETERSON

The growth of algae in pure culture has been the object of many studies (2,7). In general this work has dealt with the question of free nitrogen fixation, without taking into account the composition of the algae when grown in pure culture. In nature a profuse growth of the algae is not uncommon, and yet the algae mass which develops in this way usually is associated with various forms of life. In this report are presented the results of a study of methods for growing large quantities of algae in pure culture, and also some of the forms of nitrogen found in algae.

Technique

The method for the isolation of green algae is similar to that given in detail by SCHRAMM (7). His culture technique has been followed in this report with certain modifications. A large volume of water from the surface of Lake Mendota (100 liters) was run through a Sharples supercentrifuge at about 40,000 r.p.m. This deposit from the sides of the bowl was diluted and agar plates of the various dilutions poured. The results from the picking of single cells by means of the BARBER (1) or CHAMBERS (3) apparatus agree with those of SCHRAMM; it is a slow method without any decided advantages. After several trials of various media, the following were used (4).

1. CHODAT AND GRINTZESCO MEDIUM

Ca(NO ₃) ₂	1.0 gm.
K ₂ HPO ₄	0.25 gm.
MgSO ₄	0.25 gm.
KCl.....	0.10 gm.
FeSO ₄	Trace
Water, distilled.....	1000 cc.
Agar, washed.....	10.0 gm.
Reaction.....	P _H , 5.3-5.5

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2. MODIFIED MEDIUM

Ca(NO ₃) ₂	1.0 gm.
KH ₂ PO ₄	0.2 gm.
MgSO ₄	0.2 gm.
CaCl ₂	0.1 gm.
FeSO ₄	Trace
Water, distilled.....	1000 cc.
Agar, washed.....	10.0 gm.
Reaction.....	P _H , 7.3

A large number of plates of high dilutions were poured. The plates were inverted, placed under a belljar in a north window, and allowed to incubate for several weeks. From well isolated colonies many cultures were picked and streaked on the surface of agar slopes. From the slopes which showed a profuse growth of algae, transfers were made into glucose broth and glucose yeast water. When subjected to this test the contaminated cultures were easily detected. To secure maximum growth of algae in a short time, additions of glucose or sucrose to the CHODAT and GRINTZESCO medium were used. These carbohydrates proved very beneficial, and it was found that after 60 days incubation the sugars were almost entirely assimilated. Large amounts of the algae growth were obtained by the use of a number of tall 16 oz. flat-shaped bottles, to which 75 cc. each of liquid medium was added. After inoculation these cultures were kept in the greenhouse, flat side down. The necks of the bottles were plugged with cotton and covered with paper caps.

Experimental

GROWTH OF ALGAE IN LARGE QUANTITIES.—In connection with some studies on vitamin synthesis by micro-organisms, as well as for nitrogen analysis, a large quantity of algae was grown. A report of the vitamin studies will be given at a later date. A series of flasks and bottles with shallow layers of liquid culture media with and without glucose were inoculated with pure cultures of *Chlorella vulgaris* and *Scenedesmus quadricauda*. The plan of the experiment and the growth of the algae are shown in table I. Similar experiments were carried out with other strains of algae, but due to contamination with bacteria these were discarded. The results shown in table I

were obtained with cultures free from bacteria. At the end of the incubation period these cultures were tested for presence of bacteria. It will be seen that in the series to which glucose was added the development of the algae was far in excess of the cultures without sugar. In the absence of glucose, *Scenedesmus* grows much better than *Chlorella*. In the last column it will be seen that the algae are unusually high in mineral matter. This large percentage of ash was due in part to insoluble salts of the culture medium thrown out by the supercentrifuge. The total dry weight of the harvest was great

TABLE I
GROWTH OF *Chlorella* AND *Scenedesmus* IN LIQUID MEDIA

No.	Name	Medium	Number of cultures	Age in days	Dry weight (gm.)	Per-centage moisture	Per-centage ash
1....	<i>Chlorella vulgaris</i>	Chodat and Grintzesco	150 flasks, 100 cc. in each	128	1.03
2....	<i>Scenedesmus quadricauda</i>	Chodat and Grintzesco	200 bottles, 75 cc. in each	88	7.85	84.0	38.0
3....	<i>Chlorella vulgaris</i>	Modified plus 1% glucose	190 bottles, 75 cc. in each	20	11.3	83.0	20.8

enough to justify further work along this line; accordingly several experiments were carried out and the algae obtained saved for chemical analysis.

FORMS OF NITROGEN IN CHLORELLA.—In dissecting the forms of nitrogen present in *Chlorella* sp. grown in the absence of bacteria, the algae cells were first thrown out of the liquid medium by centrifuging, and this material scraped from the side of the bowl and dried at 65° C. When dry the material was ground to a fine powder in a mortar, and the nitrogenous constituents extracted by macerating with water, as described by TOTTINGHAM, SCHULZ, and LEPKOWSKY (9). The soluble nitrogen was separated into two portions, protein and non-protein nitrogen, by means of FOLIN and WU's (5) tungstic acid precipitation. This reagent was found by HILLER and VAN SLYKE (6) to precipitate the proteins and most of the intermediate products. Trichloroacetic acid was used for comparison, as the latter reagent was reported to precipitate the proteins and

leave the intermediate products in solution. Free and combined amino nitrogen were determined by VAN SLYKE's method before and after hydrolysis; the nitrates according to the method described by STROWD (8). The data are given in table II, and show that about 25 per cent of the total nitrogen is water soluble. About one-third of the water soluble nitrogen consists of nitrogen in the form of proteins and intermediate products, one-fourth is made up of amino nitrogen, and one-fourth is contained in peptide linkages.

TABLE II

FORMS OF NITROGEN FOUND IN PURE CULTURES OF *Chlorella vulgaris* DRIED AT 65° C., CALCULATED FOR 100 GM. ASH FREE MATERIAL*

FORMS OF NITROGEN	WEIGHT OF NITROGEN		PERCENTAGE DISTRIBUTION	
	Trichloroacetic pptn. (gm.)	Tungstic acid pptn. (gm.)	Trichloroacetic pptn.	Tungstic acid pptn.
Total nitrogen.....	7.409	7.409
Soluble nitrogen.....	1.457	1.457	23.9	23.9
1. Soluble nitrogen ppt. by reagent	0.351	0.531	5.3	8.7
2. Forms of soluble nitrogen in filtrate				
a. Free amino nitrogen.....	0.329	0.357	5.4	6.1
b. Combined amino nitrogen...	0.541	0.304	8.8	6.2
c. Nitrate nitrogen.....	0.069	0.069	1.4	1.4
d. Rest nitrogen.....	0.167	0.115	2.9	1.3

* Ash in original material 17.6 per cent.

Conclusions

1. Pure cultures of *Chlorella vulgaris* and *Scenedesmus quadricauda* were isolated from the water of Lake Mendota and grown in large quantities.
2. The nitrogen in the dried algae is largely insoluble and amounts to 7.4 per cent on the ash-free basis. About 25 per cent can be extracted with water, and is made up chiefly of protein, amino, and peptide nitrogen.

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CORRELATION AND CYCLIC GROWTH IN PLANTS

A. E. MURNEEK

Guided by the concept that fundamentally growth is a chemical process, there has come into existence a school of biologists, BLACKMAN (2), LOEB (6), ROBERTSON (12), and others, who have attempted to demonstrate that the rate of growth of an organism, when expressed as increments in volume, height, dry weight, etc., gives a curve that closely resembles and in some instances is almost identical with that of a monomolecular autocatalytic reaction. Hence growth has been considered as a catalytic process, and the organism as the end product resulting from the action of an autocatalyst on a substrate.

While these deductions have been based largely on data obtained from measurements of growth of animals, the theory has been expanded by several workers (REED and HOLLAND 11, ROBERTSON 12) so as to include plants as well. Thus the former have shown that in the case of *Helianthus*, when growth is expressed by increase of height of the stem, the measured values agree remarkably well with those calculated, when the usual formula of a monomolecular autocatalytic reaction is employed. In a recent discussion of the mechanism of growth, ROBERTSON marshalls this and similar evidence as proof of the fundamental nature of growth of all organisms.

As to the exact mechanism or the character of the essential catalyst in operation (for the type of the curve suggests such to be operative) various theories of hypothetical substances have been advanced, either catalytic, or inhibitory, or both, as responsible for the "master reaction" ("formative substances," "catalyzers," "inhibitors" of LOEB; "inhibitors" of REED; "autocatalyst" of ROBERTSON; "bios" of WILDIERS). From the present status of the question it is evident that the whole matter is still in a problematical if not hypothetical stage.

Additional information on the nature of this rather general subject has been accumulated by the writer during the past two

years, as a result of extensive investigations on growth and yield in the tomato (*Lycopersicum esculentum*). It shows that growth in at least this plant, and probably in many others, may be more readily explained on the basis of correlation. Complete evidence has been obtained that correlation is one of the most important if not the main factor concerned with the rate of growth in plants. While at present only the comparatively late stages in the development of the tomato have been taken into consideration, that is, those commonly expressed by the "autostatic" phase of the curve, striking proof has been obtained that this phase of the autocatalytic curve is produced by the effects of correlation of the fruit on the rate of growth of the main stem. It has been found that: (1) Plants grown without fruit have no such phase during the period when it normally occurs; the curve is absolutely straight. (2) The presence of fruit introduces this phase at once, the growth of the plant then running parallel with that of any normal fruiting plant. (3) The "autostatic" phase can be introduced at will by deliberately putting the plant either in a vegetative condition (flowers or fruit removed) or in a reproductive condition (normal check plants). In respect to growth, such plants during the vegetative period show a striking parallelism with strictly vegetative plants (flowers removed), and during the reproductive period with strictly reproductive plants (flowers and fruit present). (4) Normally growing plants, provided there are enough nutrients (nitrogenous) left either in the plant or the soil, will upon ripening of fruit at once commence to grow, if no other immature fruits are present, or if present are not in large numbers and not too close to the growing points. This has been found to be true in the tomato under various conditions of nutrition. It is exhibited in carbohydrate high plants particularly well; terminal growth is not only inhibited but often totally checked or destroyed by the developing fruit. Moreover, it has not been possible to disarrange the mechanism by submitting the plants to even as short an exposure to light each day as two and one-half hours. Then, too, it has been found that the effects of correlation may be more or less localized, one-half of a two stemmed plant responding to treatment, while the other half is serving as a control. Thus the evidence is clear (1) that vegetative growth in the tomato diminishes at the exact time and in exact

proportion to the amount of flowers formed and fruit set, and (2) that the rate of vegetative growth appears to be controlled by the developing fruit. The latter fact is of particular importance. It is a conception contrary to our current point of view. In the consistent gathering of evidence on the effects of vegetative growth on reproduction, we seemingly have neglected to analyze a reverse phenomenon, which is just as striking. Hence this may be a timely emphasis on the value of experimental treatment of the reproductive parts of the plant, particularly perennials, and the observation of the resultant response in both vegetation and reproduction.

That other plants behave similarly to the tomato may be gleaned from the careful analytical records on growth and yield of cotton by BALLS and HALTON (1) and by MASON (7). The extensive statistical investigations by the former show that the rate of vegetative growth in the cotton plant diminishes at the exact time and in exact proportion to the amount of flowers formed and fruit set. These results the authors unsuccessfully attempt to explain as being due to environmental factors, whereas apparently quite similar observations are correctly interpreted on the basis of correlation by MASON. In fact, MASON's experimental data and a part of his conclusions, although secured independently and based upon a different plant, are strikingly similar to those obtained by the writer. This agreement is certainly very suggestive of the importance and fundamental nature of correlation in growth of plants.

The "autokinetic" phase of the growth curve may probably find its counterpart of explanation in the influence of correlation of roots on the stem (BRENCHLEY 3, PEARSALL 9).

This evidence, although furnished largely by the tomato and to some extent by the cotton plant, tends to show that growth, as measured by increase in length of stem, is determined during the early stages probably by the development of roots and later certainly by the flowers and fruits. Hence a curve is obtained suggestive of that of an autocatalytic reaction. Moreover, such conception agrees with our present knowledge of the periodic or cyclic growth of the various parts of the body or the different organs of animals. Evidently in this respect also there is after all no fundamental difference between plants and animals. It is interesting to note that from a

mathematical interpretation of the rate of growth of corn, PEARL and SURFACE (8) conclude that there are distinct cycles of growth of the various parts of this plant, which probably are of the nature of correlations. Recently REED (10) also has pointed out that lateral growth of apricot branches is distinctly cyclic.

What, however, is the mechanism through which growth correlations find expression in the plant? Have we here evidence of localized nutrition giving rise to a metabolic gradient, such perhaps as suggested by CHILD (4)? Is it possible that sexual reproduction or gametic union may create a really extraordinary rejuvenescence in organs closely allied to the developing embryo, which results in reduction or inhibition of growth in other parts of the organism? But how is the developing embryo or fruit able to monopolize practically all of the incoming or synthesized material? Do plants have a controlling glandular organization or a system of internal secretion similar to that existing in animals? Apparently the basic part of the problem is either one of nutrition or of special secretion at certain stages of the development of the plant, leading to localized acceleration in metabolism and the rapid development of particular organs. In the absence of any proof of an internal secretory system in plants, the writer is inclined to believe that the phenomenon may be the outcome of different stages of localized nutrition, and that the carbohydrate-nitrogen relations, as proposed by KRAUS and KRAYBILL (5), may be used as a key in interpreting these rather striking conditions of growth in plants. Even if the response perchance is due to special secretion, a careful determination of some of the physiological and chemical alterations in the participating organs ought to reveal a closer picture of the true situation. With this in mind, a series of chemical analyses of various parts of the tomato plant is in progress at present.

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DISPERSION OF LIPOIDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 335

DEAN A. PACK

The dispersions of lipoids with the secession of dormancy in tissues may be of great significance and general physiological interest. The writer (3), while working on the physical and chemical changes involved in the awakening of dormant organs, found that the lipoids became dispersed as the tissues grew more active. It was further noted that the lipoids of storage and dormant cells were not so finely divided as those of actively growing cells. After a thorough rechecking of these results, the degree of lipid dispersion was studied. Other tissues were examined to determine how general such dispersion was in the physiological processes involving fats.

To the writer's knowledge, little or no work has been directed along this particular line. This fact probably explains the conflicting statements that appear in the literature. As early as 1882, SACHS (5) stated that oil drops appear in the parenchyma of root and shoot axils of *Ricinus*. WAKKER (7) in 1888 reported small oil drops evenly distributed in the protoplasm of the youngest seed tissues. He also reported that oil appeared as equally large drops in all cells. In 1900 TSCHIRCH (6) stated that the oil of seeds was not in drop form, but homogeneously mixed with the cell plasm. Three years later ANDREW (1), while working on the separation of cell constituents by centrifugation, noted oil drops in *Ricinus* seeds. He also found that growth followed by centrifuging had a tendency to separate the oil into drops. CZAPEK (2) states that the fat of dry resting seeds is amicroscopically divided in the plasma, and that oil drops in the endosperm can at no time be demonstrated. He adds that oil drops become larger and fewer as germination proceeds.

Investigation

Tissues in different stages of dormancy and growth were sectioned with the freezing microtome, stained with Sudan III or Scharlach R, examined, cleared with glycerine, and studied further. The

glycerine increases the permanency of these preparations for continued study. This material was not mounted in water, but direct in the stain and examined. Other sections were stained with osmic acid. A third lot of tissue was fixed in strong chromo-osmic-acetic solution, washed, dehydrated, imbedded in paraffin, and sectioned. Some of these sections were mounted without staining, others were stained with alcannin, while others were stained with safranin, gentian violet, and orange G. The degree of lipid dispersion was measured with the eyepiece micrometer. This was calibrated for each optic combination by the aid of the stage micrometer. Lipoid and oil are used here in their broad sense, indicating all fat staining materials

Results

Lipoid drops were demonstrated in the endosperm and embryo cells of the dry resting *Juniper* seeds. As these seeds slowly after-ripened and became more active in a germinator at 5° C., the lipid drops gradually became smaller and increased in number. This increased dispersion of the lipoids in germinated seeds over that of the lipoids in dry seeds was not due to histological treatment, since both the germinated and dry seeds received similar treatment. This was not only the case with the embryo cells, but also with the endosperm cells. The lipid drops of the hypocotyl and outer embryo cells showed a great degree of dispersion and became very numerous. This dispersion was found to precede and accompany the appearance of sugar and starch in the hypocotyl region. Further chemical analysis (4) of these seeds showed that the larger part of the sugars and all the starch appeared after germination, while microscopic study of similar germinated seeds showed that the lipoids had become well dispersed. As the seeds germinated, the lipid drops in the root and shoot axil reached a degree of dispersion equal to the limit of the microscope. During the germination period there was a decrease in the number of these lipid drops, because this material was used in the manufacture of other compounds, as the chemical analysis shows (4).

The measurements of dispersion were made by several observers and the average taken. The largest oil drops noted in the dry seeds

were $20-30\mu$ in diameter, while the smallest detectable oil drops of germinated seeds had a diameter of about 2μ . There appeared in germinated seeds other probable oil drops that were of less and less diameters, which no doubt passed the limit of the microscope.

Other plant and animal tissues were examined to see how generally this dispersion accompanied active growth and digestion. Lipoid drops in the sunflower seeds were found to become dispersed as the seeds germinated. The seeds of hawthorn, castor bean, and peach were studied with the same results. It was also noted that many actively growing and dividing animal and plant cells contained no demonstrable fat. The highly dispersed condition of the lipoids in the intestines and blood of higher animals was noted.

Discussion

The physical state of lipoids seems to depend upon the particular tissue and the condition of the tissue. Resting seeds with large amounts of lipid material often store it in the form of large drops. At times these drops are collected into masses, which fill the meshes of the protoplasm. If such tissue is stained with osmic and sectioned thickly, the whole cell is darkened and gives the impression that the fatty substance is evenly distributed throughout the cell. The massing of drops and bodies of lipoids may be distinguished in thin sections. In other instances individual drops occupy the major part of the cell cavity. Resting seeds with little fatty content deposit lipoids as fine drops or crystals. The lipoids of active cells and tissues are in a very fine state of division.

The fact that active cells and tissues contain exceedingly fine lipid emulsions or lipoids that are undemonstrable by stains, necessitates a dispersion of this lipid material at some time or other. The reasonable and actual time for this dispersion to occur is before the lipoids are decomposed, absorbed, translocated, or resynthesized. It would therefore accompany the secession of dormancy, the germination, or the activity of tissues in general.

A dispersion of the lipoids hastens these physiological processes. WENZEL's law states that the reaction velocity is proportional to the area of contact. Colloidal dispersion would increase the lipid surface area hundreds of times, and accordingly the velocity of reac-

tion. In this way the hydrolysis of lipoids is hastened. Likewise the syntheses of various lipins, lecithoproteins, and other life requiring substances are favored. The syntheses of carbohydrates from fats is accordingly accelerated. If lipid substances are hydrolyzed before absorption, or translocation, these processes are materially aided by lipid dispersion; if lipid substances are absorbed and translocated as such, they must of necessity be very highly dispersed. In either case dispersion should aid absorption and translocation. It seems that dispersion is of first importance, no matter what the following physiological changes may be.

Further physiological processes are accelerated by the action of catalysts or enzymes, whether acting chemically or physically. At the surface of all bodies there is a layer of molecules, which by their position are subjected to a new and different set of forces from those molecules in the interior of the liquid. They accordingly acquire a new set of conditions and functions. Then the colloidal particle has its absorption layer, which may be either a region of concentration or dilution. As a result, the lipid enzymes would be concentrated in particular regions and thereby hasten digestion. This concentration of enzymes must be important when one considers the enormous amount of work required of a minimum quantity of enzyme.

The enzyme or catalytic power of seeds and tissues becomes more powerful as the seeds germinate or the tissue becomes active. This increased catalytic power may be related in some way to the dispersion of lipoids and other materials. It has been found that the maximum catalase activity and the maximum lipid dispersion coincide. We should know if this is the case with the dispersion of other materials.

It may be of interest to recall the progress of events in the depositing of fatty substances in a storage organ. One first notices very fine oil droplets or bodies in the younger cells. These droplets increase in size with the age of the cells. In old cells large drops or masses of fatty substances can be demonstrated. These changes are the reverse of the dispersion of lipoids found to accompany the digestion, germination, etc., in active tissues. Just as condensation attends the storage of lipoids, so dispersion accompanies their digestion.

Acknowledgments are due to Dr. WILLIAM CROCKER, who followed this work with interest.

Summary

1. Lipoid drops were demonstrated in resting seeds.
2. The lipoids were found to disperse as the tissues became more active.
3. This dispersion reaches the limit of the microscope.
4. Lipoid dispersion is found in both plant and animal tissues.
5. A dispersion of the lipoids precedes lipid decomposition, absorption, translocation, and synthesis.
6. These physiological processes are aided and hastened by lipid dispersion.

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BRIEFER ARTICLES

EFFECT OF WOUNDING ON RESPIRATION AND EXCHANGE OF GASES

A number of references on the effect of wounding on respiration occur in the literature. PALLADIN¹ refers to one experiment in which 300 gm. of uninjured potato tubers produced from 1.2 to 2 mg. of carbon dioxide per hour. After the rate had been determined, each tuber was quartered, and the pieces were left at the same temperature and in the same surroundings as before. The rate of respiration increased up to the twenty-eighth hour after cutting, when it was 18.6 mg. per hour; then it began to decrease.

MAGNESS² has studied the effect of wounding on the internal atmosphere of apples. A thin slice of peel was removed from each end of the fruit, and they were then put in storage at the various temperatures, by the side of the whole fruit serving as checks. The data obtained showed that removing the epidermis greatly facilitates the entrance of gases to the tissues, and also the escape of accumulated carbon dioxide. MAGNESS remarks that it would be interesting to know to what extent increased respiration following wounding is due to mechanically facilitating this gaseous exchange, and to what extent it is due to actual metabolic changes in the wounded tissues.

Experiments by STICH³ in 1891 showed that when 65.3 gm. of potato tubers were quartered, and bound together again and sealed, their rate of respiration increased from 4.3 mg. per hour before injury to 9.5 mg. after wounding; while another lot which was quartered but left free in the respiratory apparatus respired at the rate of 25.7 mg. per hour. These data indicate that 120.9 per cent of the increase in the rate of respiration is due to injury, while 497.6 minus 120.9, or 376.7 per cent of the increase in the later case, is due to facilitating the exchange of gases.

¹ PALLADIN, V. I., *Plant physiology*. 2d Eng. ed., edited by B. E. LIVINGSTON (p. 213).

² MAGNESS, J. R., Composition of gases in intercellular spaces of apples and potatoes. *BOT. GAZ.* 70:308-316. 1920.

³ STICH, C., Die Athmung der Pflanzen bei Verminderter Sauerstoffspannung und bei Verletzungen. *Flora* 74:1-57. 1891.

Some experiments with wounded and uninjured sweet potatoes contribute further data on this problem. By means of a cork borer, cylindrical plugs were removed from one lot, producing an injured surface area of 7385.3 sq. mm. The rate of respiration increased from 64.27 mg. per kilogram hour before injury to 126.81 mg. per kilogram hour after injury. From a similar lot plugs were removed but replaced and sealed, producing an injured area of 7636.4 sq. mm. In this case the rate of respiration increased from 60.06 mg. per kilogram hour before injury to 70.36 mg. after wounding. Therefore the rate of respiration increased 97.3 per cent when the injured area was exposed, while it increased only 17.15 per cent when not exposed. It is evident that such increase is largely due to mechanically facilitating the exchange of gases, rather than to direct wound stimulation.—G. R. JOHNSTONE, *University of Southern California, Los Angeles, Cal.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Self-sterility.—The bulk of investigation of this problem has been about as sterile as the material used. From the viewpoint of genetics, however, there is one set of experiments that has repeatedly borne fruit of fascinating quality. These are the experiments upon *Nicotiana*, which have been continued for a number of years by EAST and his associates.¹ The gross physiology of self-sterility has clearly been shown to be a matter of pollen tube growth. Own pollen tubes exhibit a slow and steady rate of growth which ordinarily does not permit fertilization to occur within the life of the flower; while foreign pollen tubes grow at such a rapidly accelerated rate as to bring successful fertilization.

The basic genetical problem of self-sterility was rather readily solved, for self-sterility was found to be a Mendelian recessive in crosses between self-sterile and self-fertile species. A secondary problem was presented by the relation of self-sterile plants inter se, and this proved to be considerably more involved. In the earlier experiments the self-sterile plants were found to be completely cross-fertile; that is, every self-sterile individual was fertile in crosses with every other self-sterile individual. This was accounted for by assuming that every one of these individuals differed somewhat from every other, and this difference brought cross-fertility. Presumably then, two individuals of identical genetical constitution would prove cross-sterile. To test this hypothesis the attempt was made to obtain groups of individuals of the same genotype. This, of course, could be accomplished if self-sterile individuals could be

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inbred, an act which would seem by definition to be impossible. Since self-sterility, however, was merely the result of the slow growth of own pollen tubes, one might hope to bring about self-fertilization by giving the own pollen tubes an abnormally early start. As a matter of fact, pollination in the very young bud did bring the desired result; self-sterile individuals were thus inbred. The progeny so obtained (in a few generations) fell into several groups, such that every individual of a group was cross-sterile with every other individual of that same group, although cross-fertile with individuals of other groups. Thus cross-fertility was shown to be due to genetic difference.

Following this, the interrelationship of these various sterility groups was examined critically, and a surprising genetic phenomenon brought to light. For example, in a family composed of three such intra-sterile groups, X, Y, and Z, the following breeding results were obtained. X female mated with Y male gives, in equal numbers, progeny of types Y and Z, but none of X. Y female with X male gives progeny of X and Z, but none of Y. Similar results are obtained from reciprocal crosses of Y and Z and X and Z. In short, two classes always appear in equal numbers, but the class of the mother is never represented.

These surprising results, taken together with certain others which are more involved but increasingly convincing, support the following interesting genetical interpretation. The sterility phenomena are governed by a series of three multiple allelomorphs, S_1 , S_2 , and S_3 . Sterility group X is of constitution S_1S_3 , Y is S_1S_2 , and Z is S_2S_3 . The X female, S_1S_3 , stimulates only those tubes containing the allelomorph she lacks. Mated with a Y male, she would be presented with pollen tubes carrying S_2 and others carrying S_3 . Only the latter are successful; hence the only progeny produced are S_1S_2 (Y) and S_2S_3 (Z). Comparable results are produced in the other possible reciprocal crosses.—M. C. COULTER.

Taxonomic notes.—CHODAT,² in continuation of his studies of the alpine flora of Switzerland (Grand St. Bernard), has published an account of 7 genera, including a new genus (*Bernardium*) and new species in *Anabaena* and *Glenodium*.

YAMAMOTO³ has published a new genus of Orchidaceae from Formosa, under the descriptive name *Tuberolabium*.

HONDA,⁴ in his revision of the grasses of Japan, appearing in a series of contributions, has now included 83 species. In the last four contributions 54 species are presented, including a new genus (*Eulaliopsis*), 7 new species, and many new varieties.

² CHODAT, R., Algues de la région du Grand Saint-Bernard. Bull. Soc. Bot. Genève. pp. 33-48. 1924.

³ YAMAMOTO, Y., Genus novum Orchidacearum ex Formosa. Bot. Mag. Tokyo 38: 209-212. 1924.

⁴ HONDA, M., Revisio Graminum Japoniae. IV-VII. Bot. Mag. Tokyo 38: 49-59, 110-120, 189-201. 1924; 39: 33-43. 1925.

KOIZUMI,⁵ in his investigation of the flora of Oriental Asia, has published 61 new species, including a new genus of Liliaceae (*Brachycirtis*) and of Gramineae (*Chikusichloa*).

BRAINERD⁶ has published a full and well illustrated account of 82 natural violet hybrids. The plants studied have been secured from all parts of the United States and some provinces in Canada, transplanted at Middlebury, Vermont, and kept under experimental control. The results and conclusions are of great interest in connection with the problems of inheritance and limitations of species.

McNAIR⁷ has investigated the taxonomy of "poison ivy" in great detail, and has reached the conclusion that many of the new species proposed during recent years are synonymous. He recognizes only four species of *Rhus* included in the poison ivy group, and has described them in detail, with full synonymy and collections. This certainly reduces a very long list of specific names to synonymy. The species recognized are *R. quercifolia*, *R. diversiloba*, *R. Toxicodendron*, and *R. divaricata*.

NANNFELDT⁸ has revised a section (Asiaticae) of *Centella*, a tropical genus of Umbelliferae, recognizing 11 species, 3 of which are described as new, and 4 transferred.

FITZPATRICK⁹ has made a critical study of the Pyrenomycete genus *Fracchiæa* occurring on branches of many trees, recognizing 16 species.

ST. JOHN and PARKER¹⁰ have described a new subgenus (*Altericarex*) of *Carex*, distinguished by having four stigmas and a 4-angled achene, which includes the new section Tetragonae, containing one species (*C. concinnoides*) of western North America.—J. M. C.

Evaporation and transpiration.—A few years ago BATES¹¹ devised a metal atmometer for use in forest studies that resembled somewhat a foliage leaf in its structure. It had an impervious upper surface and a perforated lower surface with porous moist material placed between. Experiments showed that the losses

⁵ KOIZUMI, G., Contributiones ad cognitionem florae Asiae Orientalis. Bot. Mag. Tokyo 38:87-113. 1924; 39:1-30. 1925.

⁶ BRAINERD, E., Some natural violet hybrids of North America. Vt. Agric. Exp. Sta. Bull. 239. pp. 205. pls. 82. 1924.

⁷ McNAIR, J. B., The taxonomy of poison ivy. Field Mus. Nat. Hist. Bot. Series 4:55-70. 1925.

⁸ NANNFELDT, J. A., Revision des Verwandtschaftskreises von *Centella asiatica* (L.) Urb. Svensk Bot. Tidskrift 18:397-426. 1924.

⁹ FITZPATRICK, H. M., The genus *Fracchiæa*. Mycologia 16:101-114. 1924.

¹⁰ ST. JOHN, H., and PARKER, C. S., A tetramerous species, section, and subgenus of *Carex*. Amer. Jour. Bot. 12:63-68. 1925.

¹¹ BATES, C. G., A new evaporimeter for use in forest studies. Mon. Weather Rev. 47:283-294. 1919.

from this instrument followed the transpirational losses from small coniferous trees rather closely. The inventor made no further claims that it would measure the transpiration from these or other plants. He evidently intended that it should be used as an instrument that would give some approximate determination of the environmental conditions that affect the rate of water loss by vegetation; in other words, it would measure what has been called the "evaporating power of the air."

A different understanding has led SINGH¹² to make some comparisons between rates of evaporation from a shallow open pan and from Bates atmometer and the transpiration losses from a jasmine plant. He obtained results very similar to those formerly obtained by SHANTZ for alfalfa. He naturally reaches the conclusion, already held by practically all ecologists, that there are regulatory factors that affect water losses from plants that do not influence the losses from atmometers or evaporimeters.

Another recent study by DOLE¹³ of some of the conditions that determine variations in transpiration and evaporation has shown that the previous environmental history of the plant affects its rate of water loss, as does the amount of available soil water, indicating that in critical studies of transpiration a controlled environment is imperative. He further shows that in an unchanging controlled environment the phenomena of transpiration and evaporation are closely parallel. This he takes to mean that deviations from the same tendencies in the two phenomena in a varying environment are due chiefly to the secondary effects of the environment upon the organism, and to the changes within the organism thus induced. DOLE's report is to be commended for its care in the analysis and control of environment, and for the carefully considered conclusions.—GEO. D. FULLER.

Illinois State Academy of Science.—The *Transactions* for the 1924 meeting (vol. 17. pp. 444) include the following papers of botanical interest: Plant communities of Glacier National Park, W. G. WATERMAN; A new mushroom (*Marasmius nucicola*), W. B. McDOUGALL; Presence of living organisms in lake ice, S. EDDY; Some outstanding features of the plant disease situation in Illinois during 1923, L. R. TEHON; Some north and south stream valleys in Illinois and their vegetation, G. D. FULLER and C. J. TELFORD; Preliminary check list of the vascular plants of the Illinois State Park at Starved Rock, F. THONE; Forest preservation, E. M. NORTH; The cotton industry of Southern Illinois, F. H. COLYER.—J. M. C.

¹² SINGH, B. N., On the use of the Bates evaporimeter and evaporimeters in general in studies on plant transpiration, especially in the open air. Jour. Ind. Bot. Soc. 4: 149-179. 1924.

¹³ DOLE, E. J., Studies on the effects of air temperature and relative humidity on the transpiration of *Pinus Strobus*. Vermont Agric. Exper. Station Bull. 238. pp. 38. figs. 6. 1924.

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PHYSIOLOGICAL STUDY OF THE SYMBIOTIC GERMINATION OF ORCHID SEEDS

LEWIS KNUDSON

Introduction

In two previous papers (6, 7) I have presented evidence that germination of orchid seeds is dependent upon an available supply of organic matter. These papers emphasized the nutritional aspects of the problem, and also presented certain views with respect to the possible function of the fungus in the pure culture experiments made by BERNARD (1), BURGEFF (4), and others. All who have studied the germination of orchid seeds agree that there is something very unusual about the seeds of most orchids, for they cannot germinate when merely supplied with water and nutrient salts. According to BERNARD, BURGEFF, RAMSBOTTOM (11), and CONSTANTIN and MAGROU (5), normal germination is dependent on infection of the embryo by the appropriate fungus. Symbiosis is believed by these men to be obligative for the normal development of orchids.

Symbiosis in a broad sense means the living together of two dissimilar organisms. Under this definition, a very wide range of associations might be considered. The term as applied to the relation between the orchid and the orchid fungus includes the idea that the fungus is in some manner of benefit to the orchid plant, and, according to BERNARD, BURGEFF, and others, germination is normal-

ly induced only after the embryo becomes infected. The experiments first made by BERNARD and later by BURGEFF were so striking, and to many seemed so conclusive, that obligative symbiosis has been accepted as established for orchids.

According to BERNARD, BURGEFF, and RAMSBOTTOM, the embryo becomes infected, and provided that the infection is confined to the lower part of the embryo, germination will take place. The explanation offered is that the fungus increases the concentration of the cell sap, and this increase in concentration acts as a physical-chemical stimulus to germination. BERNARD suggests that this increase might be brought about by the digestion of starch within the embryo, this digestion being effected by enzymes excreted by the fungus. He recorded experiments in which germination was obtained by the use of higher concentrations of the nutrient solution. This nutrient contained an extract of the tubers of a certain orchid. In discussing these experiments, BERNARD did not emphasize the nutritional aspects of the problem, but stated that the higher concentration was a physical-chemical stimulus to growth, and that one might compare the action of the fungus with the growth stimulus imparted to an egg by the spermatozoid. Furthermore, he stated that the stimulus to growth induced by the high concentrations of organic substances is comparable with the influence exerted by chemical or other treatments in inducing the development of an unfertilized egg. BERNARD concluded that the stimulus acts to increase the formation of absorbing hairs, and with the increased concentration of sap there is likewise an increase in the intake of water. BURGEFF, while differing somewhat in an explanation of the function of the fungus, expresses the view that both fungus and orchid are benefited by the association.

The evidence presented by adherents of the symbiotic view of germination may be summarized as follows: (1) the roots of orchids are generally infected by a characteristic fungus which is considered non-injurious; (2) different genera of orchids may have different strains or species of this fungus; (3) seeds sown under pure culture on various culture media, especially those media containing starch or other insoluble organic matter, do not germinate except when the fungus is present; (4) germination apparently is induced by some

strains of the fungus and not by others; (5) there must be maintained a definite balance between fungus and host; (6) while it is recognized that germination of orchid seeds may be obtained by the use of sugars in the culture medium, germination under these conditions is abnormal, and not of course common in nature.

In my first paper (6) considerable evidence was presented relative to the use of sugars and plant extracts in inducing germination. In a second paper (7) I extended these studies to include seeds of various orchid genera, and brought out other salient facts relative to the subject of symbiosis in orchids. In addition to the experimental evidence on the nonsymbiotic germination of orchid seeds, I included a critical discussion of the evidence presented by BERNARD and BURGEFF on the necessity of the fungus. I admitted at that time that certain evidence presented by these two investigators was difficult of interpretation except on the basis that the fungus was essential. The validity of the fungus hypothesis was questioned very largely on the basis that BERNARD and BURGEFF had failed to take into consideration the effect of chemical changes in the external medium on germination. Both used organic matter in the culture medium. In some cases starch was used. In BERNARD's experiments salep was used, which is really pulverized tubers of a certain orchid. This latter substance contains some starch, pentosans, some sugar, and other organic substances.

These facts are important because of the well recognized ability of fungi to secrete enzymes which are capable of digesting a variety of substances. There is still another possibility that was ignored by the proponents of the symbiosis hypothesis, which is that substances secreted by the fungus or produced on autolysis of the fungus may be involved in the germination. In my first paper I stated as a possible explanation of the favorable effect of the fungus, that "It is conceivable that the germination is induced not by any action of the fungus within the embryo, but by products produced externally by digestion or secreted by the fungus." I also stated that the evidence as regards the necessity of the fungus was not yet proved, and that considerable work was yet to be done before the validity of the fungus hypothesis could be proved or disproved.

Another phase of the problem was presented by BERNARD,

namely, the loss by the fungus of its capacity to induce germination after prolonged culture in the laboratory. I stated: "It is entirely possible that there has been no loss in the fungus, but at the time of inoculating the culture, the physiological state of the embryos was such as to resist or permit of infection. Those in which the infection was confined to the lower cells could still germinate despite the fungus. Those invaded to a greater extent would be killed. These and other experiments suggest that one of the causes of failure of germination is the parasitic character of the fungus."

In planning these experiments on the relation of the endophytic fungus to germination, the questions that arose directly from a consideration of the problems were these: Does the fungus change starch to sugar? Is there any secretion by the fungus? Are the embryos that continue to grow always invaded? Are the embryos ever killed by the fungus? Is there any relation between the time of infection and the degree of injury or rate of germination? Are the external changes induced by the organism sufficient to produce germination? Are there any other fungi capable of inducing germination? Is there a change in the "activity" of the fungus when grown under pure culture conditions? Is the fungus equally effective in inducing germination under conditions of varying concentration of starch? Why is germination of *Cattleya* not possible with the fungus from *Odontoglossum*? These questions are considered in the experiments which follow. The evidence presented by these and various other experiments leads to but one conclusion, which is that the fungus is not necessary for germination, at least for seeds of *Cattleya*.

Methods

Except where otherwise indicated, the culture methods were essentially those described in my first paper. For the most part the culture vessels were culture tubes 200 mm. \times 20 mm. Whenever the hydrogen ion concentration was to be controlled, the tubes were of Pyrex glass. The nutrient solution used for the most part was solution B of my first paper, although in certain experiments the dibasic potassium phosphate K_2HPO_4 was replaced by the monobasic phosphate KH_2PO_4 . The reason for this change was to afford a

slightly more favorable hydrogen ion concentration. Solution B unmodified has the following composition:

Ca(NO ₃) ₂	1 gm.
K ₂ HPO ₄	0.25 gm.
MgSO ₄ NH ₂ O.....	0.25 gm.
FePO ₄	0.05 gm.
(NH ₄) ₂ SO ₄	0.50 gm.
Distilled water.....	1000.00 cc.

To this nutrient solution was added agar at the rate of 1.75 gm. for each 100 cc., and sugar or starch as the experiment required. The culture medium was heated to "dissolve" the agar, and then 10 cc. of the solution added to each tube. The tubes were plugged with cotton, autoclaved at 15 pounds pressure for twenty minutes, and then sloped. Each tube was then capped with a glass vial to decrease the possibility of spores lodging on the cotton and growing through it or between the cotton stopper and the side of the tube.

The seeds were sterilized by the use of calcium hypochlorite (13), using the filtered solution obtained by shaking 10 gm. of calcium hypochlorite in 150 cc. of water. They were then placed in a small tube about 60 mm. × 6 mm., and the hypochlorite added. The tube was then shaken until each seed became moistened, and after five or ten minutes in this solution the seeds were sown by means of a looped platinum needle, employing the usual bacteriological technique to prevent contaminations. After a few minutes in the hypochlorite most of the seeds accumulate on the surface of the solution, and are planted by means of a platinum loop without any previous rinsing. All the cultures were maintained in a small glass compartment in the greenhouse. No attempt was made to maintain a constant temperature. In the summer the tubes were shaded from direct sunlight. In general the temperatures prevailing were 20°–30° in the daytime and somewhat lower at night, except for marked departures during the spring and fall, when the houses were not heated.

Hydrogen ion determinations were made of the culture media, both inoculated and uninoculated. For this purpose a definite quantity of either methyl red or brom thymol blue was added to

the culture solution. To distribute the indicator throughout the agar mass, the tube was heated or left standing over night, by which time the indicator was fairly well distributed. Generally the tube was heated. On cooling, the color was compared with standard buffer solutions containing a like quantity of the desired indicator. The turbidity of the agar was compensated by interposing another tube of agar between the buffer solution and the source of light. In some of the experiments it was essential to adjust the hydrogen ion concentration of the culture solution. This was accomplished by first making up the solution with agar. This was then autoclaved and to this was added 0.1 normal HCl until the desired reaction was obtained.

Sugar determinations of the culture media containing starch were made by extracting the culture medium with 95 per cent alcohol. To the culture tube 10 cc. of 95 per cent alcohol was added. This was then heated in the autoclave, and when the contents of the tube was liquefied it was poured into a beaker containing a volume of alcohol ten times that of the original tube content. The alcohol precipitated all starch and agar. The alcohol extract was then filtered from the residue, and the residue washed with alcohol. The alcoholic extract was then evaporated to dryness in a water bath, and this residue redissolved in 25 cc. water. Sugar was determined by the Walker-Munson method for glucose. When sucrose was used the procedure was the same, but the reducing sugar is expressed as invert sugar.

In making growth measurements of these minute objects, the diameter of the embryo or protocorm of the seedling was measured. Measurements were made by the use of a microscope, using an ocular micrometer. The diameter of the embryo is not an exact measure of growth, since the embryos of two different culture media may not be of the same shape. The embryos at first are somewhat oval, later they are spherical, and still later they may become somewhat top-shaped. It would be an extremely tedious task to determine the volume of the seedling, and from most of the data here recorded the volume may be calculated from the diameter, since the embryos measured were spherical or nearly spherical in shape. This is true except for those embryos having a diameter of less than

225 μ . Each average figure given for the diameter measurements in nearly all cases is the average obtained from measurements of fifty individual embryos.

In studying the infection of the embryo by the endophytic fungus, it was found essential to prepare the specimens for microscopic examination. For this purpose the embryos were fixed in a chromo-acetic fixative and carried through the preparatory stages for imbedding in paraffin. For the most part fixation was good. The imbedded material was sectioned generally about 15 μ in thickness and then stained with Haidenhain's iron-alum haematoxylin stain. Various other stains were used, but the latter proved excellent as a stain for the hyphae. In using this stain the material was first overstained and then destained. The fungus retained the stain more markedly than the host tissue and protoplasm; consequently differentiation was quite marked.

Isolation of fungus

The isolation of the orchid fungus gave considerable trouble, and various attempts to isolate it resulted only in the isolation of a *Fusarium* and other fungi, some of which were quite destructive to the embryos. One of the causes of this failure was that the isolation was attempted from portions of the root which appeared most heavily infected, and which generally were far removed from the root tip. It would appear from such results that in the older portions of the roots the orchid fungus is no longer alive, and that secondary infections have occurred. There is evidence for this also in the fact that complete disintegrations of the roots of *Cattleya* and of other orchids are commonly observed.

In selecting roots for isolation of the fungus, therefore, healthy appearing roots only were selected, which were not at all discolored, but had a glistening whitish appearance. From such roots freehand sections were made, beginning at the root tip and continuing back from the tip until infected cells were found in the section. From this newly infected root tissue the isolation was made. The roots were thoroughly cleaned in running water and by means of a soft brush. They were then immersed for a few minutes in a solution of calcium hypochlorite made by shaking 10 gm. of the hypochlorite

in 150 cc. of distilled water, then filtered, and afterwards rinsed in sterile distilled water. The roots were then momentarily flamed, and by means of a scalpel and forceps the outer velamen was peeled off. The root freed of velamen was again passed through a flame very quickly. By means of a sterilized razor blade, the root was then cut into sections about 1 mm. in length, and one or two of the sections placed on the culture medium in a Petri dish. The culture medium used was solution B + 0.5 per cent starch. Out of sixty-five plates made, twenty showed no growth at all; *Fusarium* and a few other fungi appeared in about twenty-five plates; in the remainder was noted a rather slow and weak growing fungus which was isolated. These appeared microscopically and macroscopically to be identical. The plant from which the roots were obtained was a vigorous growing plant of *Cattleya Portia*.

Using a slightly different procedure, DIXON, working under my direction, isolated an organism resembling that of BERNARD, and which proved later to be identical with the fungus which I isolated.

Influence of fungus on germination

EXPERIMENT 1.—Having isolated a fungus which morphologically fitted the description given by BERNARD for the fungus of *Cattleya* (*Rhizoctonia repens*), it was first necessary to prove the fungus the correct one by inoculation experiments, and to determine its ability to induce germination. The criterion of the true fungus, as RAMSBOTTOM states, is that it will induce germination. Some preliminary experiments gave positive results, although the percentage of germination was small.

A more elaborate experiment was devised, therefore, to determine the ability of the fungus to induce germination in various nutrient media, and at the same time to determine whether the fungus would have any influence on the chemical composition of the nutrient medium. If the fungus digested the starch of the nutrient medium, changing it to sugar, then the necessary condition would be provided for germination, and this fact would need to be taken into consideration. The results are given in table I. Three different nutrient solutions were used, and to each was added 1.5 per cent agar and starch. The solutions used were solution B as previously

TABLE I
SEEDS SOWN SEPTEMBER 16, 1922; NOTES MADE APRIL 12, 1923

Culture no.	Date of inoculation	Percentage germination	Percentage dead	Average diameter (μ)	P_H of culture solution	Mg. of reducing sugar	General condition
PEPPER'S CULTURE MEDIUM							
PA 3.....	September 16	13	9.0	506	4.6	Light green; one leaf produced
PA 4.....	September 16	9	20.0	447	4.6	95.3*	Light green; one leaf produced
PA 7.....	September 28	65	1.5	651	4.6	Dark green; one and two leaves
PA 9.....	Not inoculated	0	100.0	179	7.0	Brown and apparently dead
PA 11.....	Not inoculated	0	100.0	151	7.0	5.4*	Brown and apparently dead
PA 12.....	Not inoculated	0	100.0	165	7.0	Brown and apparently dead
SOLUTION B							
BA 4.....	September 16	0	90.0	317	4.0	170	{ Few with leaves dark green; others still alive, light green; others brown and apparently dead
BA 5.....	September 28	2	80.0	380	4.0		
BA 7.....	September 28	0	100.0	359	4.0		
BA 8.....	September 28	0	100.0	279	4.0		
BA 9.....	Not inoculated	0	100.0	172	6.0	Brown and apparently dead
BA 10.....	Not inoculated	0	100.0	161	6.0		
BA 11.....	Not inoculated	0	100.0	162	6.0		
SOLUTION D							
DA 2.....	September 16	37	1.5	648	181*	Brown and dead
DA 3.....	September 16	60	4.0	767	4.8		
DA 4.....	September 16	0	53.0	588		
DA 5.....	September 28	25	1.0	648	4.8		
DA 6.....	September 28	18	2.0	641		
DA 7.....	September 28	0	33.0	581	4.8		
DA 8.....	September 28	20	6.0	679		
DA 9.....	Not inoculated	0	100.0	150	6.3		
DA 9.....	Not inoculated	0	100.0	131	6.3		

* Total amount of sugar in three tubes.

described, Pfeffer's solution, and a third designated solution D. The formulas for the two latter solutions are:

Pfeffer's solution		Solution D	
Ca(NO ₃) ₂	4 gm.	CaCO ₃	2 gm.
K ₂ HPO ₄	1 gm.	K ₂ HPO ₄	1 gm.
MgSO ₄ ·7H ₂ O.....	1 gm.	NaCl.....	1 gm.
KNO ₃	1 gm.	(NH ₄) ₂ SO ₄	2 gm.
KCl.....	0.5 gm.	NH ₄ Cl.....	0.5 gm.
FeCl ₃	0.040 gm.	Tap water.....	1 liter
Distilled water.....	5 liters	Potato starch.....	0.6 per cent
Soluble starch.....	0.5 per cent		

An examination of table I brings out a number of significant facts. The fungus induced germination, but in no case was 100 per cent germination attained at the time of taking the final observations. In cultures PA 3, 4, 7, and DA 2 to 8, a greater percentage of germination would have been obtained had the culture been left in the greenhouse a few weeks longer. The fungus isolated not only fitted the description given by BERNARD regarding its morphological characteristics, but likewise induced germination. In the absence of the fungus no germination occurred, and the growth made by the embryos was characteristically slight. From these and other experiments later described there is abundant evidence regarding the effectiveness of the fungus in inducing germination.

What is the explanation of the stimulating action of the fungus? As shown by table I, in the inoculated cultures the hydrogen ion concentrations for the PA cultures are equivalent to P_H 4.6, while in the uninoculated cultures the values are P_H 7.0. Likewise the values are P_H 4.0 and P_H 6.0 for the BA series, while for the DA series the values are P_H 4.8 for the inoculated cultures and P_H 6.3 for the uninoculated. Here is evidence that the hydrogen ion concentration is markedly increased by the fungus, and this must be due to organic acids produced and excreted by the fungus.

It will be noticed that the glucose content for the inoculated cultures is considerable, while in the uninoculated cultures there is practically no sugar. The small amount present probably is due to reduction by dextrins which were not precipitated by the alcohol, or to slight hydrolysis of some of the dextrin on heating the tubes. This shows that the fungus has digested starch, converting it to

sugar, and in its metabolism some of its sugar is changed to some organic acid. Thus in cultures PA 3, 4, and 7 the total sugar found was equal to 95.3 mg., or 31.7 mg. per tube. The original amount of culture medium added was 10 cc. to each tube, so that on this basis the concentration would equal 0.3 per cent sugar. At the close of the experiment, however, due to evaporation, there was present only 6 cc. of the culture medium, so that the final concentration was equal to 0.5 per cent sugar.

In cultures BA 4, 5, 7, and 8 the total sugar was 170 mg., or 42.5 mg. per tube. On the basis of 10 cc. per tube the initial concentration of sugar would be 0.4 per cent, but on the basis of 6 cc. the final concentration was about 0.7 per cent sugar. In DA 4, 5, and 7 there was present about 60 mg. of sugar per tube. The concentration of sugar, therefore, was nearly twice that of cultures PA 3, 4, and 7. This probably is the reason of the greater growth in the DA series.

The studies made by NANZ in this laboratory (not yet published) show that at a hydrogen ion concentration of $P_H 6$ growth is slow, even though sugar is provided; but at a hydrogen ion concentration of $P_H 4.7$ to $P_H 5.2$ growth is very much accelerated. At the higher P_H value the embryos are yellowish or even whitish in color, but at the higher hydrogen ion concentration (lower P_H) the embryos become deep green. The presence of sugar and the favorable hydrogen ion concentration are altogether adequate to explain the germination, without ascribing it to any internal action of the fungus.

Another interesting aspect of the fungus relationship is developed by the data in table I. It may be noted that in Pfeffer's solution only a relatively low percentage of the embryos were killed. With but two exceptions this is likewise true for the DA cultures. On solution B, however, a rather high percentage of the embryos in the inoculated cultures were killed. These data suggest that the degree of infection of the embryo depends upon the nutrient solution used, which in turn affects the physiological state of the embryo.

Slides were made of material from all these cultures, following the methods previously described. Microscopic examination of serial slides of individual seedlings of the inoculated cultures of series PA revealed some interesting facts. Not all of the seedlings

were infected by the fungus; some were markedly infected and others but slightly so. In the latter case the fungus was confined to one or two cells at the base of the protocorm. The fact that not all the seedlings were infected is significant, for if some develop without being infected, then it is apparent that the stimulative effect of the fungus is not internal, but is due to chemical changes in the culture medium. Results of like character were obtained with material of the other series.

EXPERIMENT 2.—In this experiment the methods used were essentially the same as those of the preceding experiment. Solution B was used, modified by substituting KH_2PO_4 for K_2HPO_4 , and 0.25 per cent soluble starch instead of 0.5 per cent. The KH_2PO_4 was used to give initially a more favorable hydrogen ion concentration. The lower concentration of starch was used because BURGEFF used concentrations close to this value in some of his experiments. These cultures were kept in the laboratory three or four days before they were taken to the greenhouse. Detailed data are given in table II. These data indicate that the fungus was favorable to the germination of some of the seeds, but most of them were killed. A few of those living had produced leaves, but not all were green, indicating the pathogenic character of the fungus. Ninety per cent of the seeds were killed in the inoculated cultures, while in the control cultures the embryos were still living, but of course had made but little growth. Furthermore, in the cultures RA 11, 12, 15, and 16 the large living embryos or seedlings were infected but slightly, while most of the smaller embryos which had been killed were completely invaded. The explanation for the high mortality must be ascribed to the low concentration of starch and a consequently low concentration of sugar.

EXPERIMENT 3.—In view of the fact that so many seeds were killed by the fungus in experiment 2, and since the results were so unsatisfactory from the standpoint of favorable germination, another similar experiment was made. Some of the evidence in table I indicating that less injury resulted if the cultures were inoculated some days after sowing, it was decided to inoculate the cultures some time after the date of planting. One series was inoculated a week after planting, while the second series was inocu-

lated nearly six weeks after planting. The observation made by BERNARD and BURGEFF that the fungus produced a marked acceleration in growth of the embryo was noted in this and other experiments. The data appear in table III.

TABLE II

Cattleya HYBRID SEEDS PLANTED AND CULTURES INOCULATED DECEMBER 21, 1922;
NOTES MADE JUNE 10, 1923

CULTURE NO.	PER- CENTAGE DEAD	AVERAGE DIAM- ETER (μ)	OTHER OBSERVATIONS	P _H OF CULTURE SOLUTION	GLUCOSE PER TUBE (MG.)
Solution B + $\frac{1}{4}$ starch					
RA 1.....	0	190	All green	5.6	1.2
RA 3.....	0	193	All green	5.6	0.8
RA 7.....	0	190	All green	5.6	0.8
Solution B + $\frac{1}{4}$ starch inoculated					
RA 15.....	90	315	5 per cent green, 5 per cent albino	4.2	9.4
RA 11.....	90	432	5 per cent green, 5 per cent albino	4.2	7.4
RA 12.....	80	423	10 per cent green, 10 per cent albino	4.2	8.8
RA 16.....	85	432	6 per cent green, 9 per cent albino	4.2	7.2
RA 17.....	95	433	5 per cent green	4.2	7.5

TABLE III

Cattleya HYBRID SEEDS PLANTED MARCH 15, 1923; CULTURE SOLUTION B + $\frac{1}{4}$
PER CENT STARCH

DATE OF INOCULATION	AVERAGE DIAMETER OF EMBRYOS (μ)			OBSERVATIONS MADE JUNE 15
	April 12	May 16	June 15	
Not inoculated.....	126	162	180	Light green; 3 per cent dead
March 22.....	171	243	288	Dark green; 3 per cent dead
April 27.....			252	Dark green; 3 per cent dead

On October 3 additional notes were taken on this series of cultures. Those cultures inoculated on March 22 had embryos which averaged 315μ in diameter, while those inoculated on April 27 were 414μ in diameter. Those of the control series averaged only 220μ in diameter. In each of the inoculated cultures, 10 to 15 per cent of the embryos had developed to a well defined seedling stage,

each seedling having one or two leaves. The remainder were just about to produce the "leaf point." On this same date the culture solutions were analyzed for sugar, and hydrogen ion concentrations made. The control cultures (not inoculated) gave readings of P_H 5.7, while the inoculated cultures were P_H 4.4. Starch tests were likewise made. All the starch had been digested in the inoculated cultures, while in the uninoculated cultures starch had not been changed. Sugar determinations were also made. The inoculated cultures yielded 2-2.4 mg. glucose, while the uninoculated cultures yielded 0.3 mg. sugar.

The data in the table and those obtained on October 3 show the favorable effect of the fungus on germination. In this experiment, however, there was no killing of the seeds. This is due either to the fact that the cultures were inoculated some time after planting, or to inherent resistance on the part of the seed to the fungus, and perhaps also to the fact that the cultures were taken immediately to a well lighted greenhouse in March.

The fact that all the starch was digested by the fungus, and that sugar was produced, together with the production of a favorable hydrogen ion concentration, constitutes evidence that external changes have taken place, and, as in the preceding experiment, these changes are sufficient to cause germination.

The objection might be made that the amount of sugar in these cultures is too little to be of much significance, but it should be taken into consideration that these analyses were made nearly six months after the date of inoculation, and that higher concentration of sugar prevailed earlier in the experiment. The fungus, of course, would use sugar in growth, and even after growth some sugar would be used. This leads to another point, that in the inoculated tubes there is probably an increase in the carbon dioxide content of the air of the tubes. This increased CO_2 content, increased sugar, and higher hydrogen ion concentration would suffice to explain the accelerated rate of germination.

Regarding infection, prepared slides were carefully examined. Those inoculated on March 22 were infected by the organism, and some small ones were completely invaded. There were very few completely invaded, however, and the mortality was slight. While

individual embryos and seedlings were not sectioned, it was possible to examine the sections serially by the use of a mechanical stage. Some of the seedlings in those cultures inoculated on March 22 were not infected, and more appeared not to be infected in those inoculated on April 27.

When starch is provided as the organic matter in the culture media, there is no germination unless the fungus is supplied. The explanation of the action of the fungus cannot be ascribed to any internal changes induced by the fungus, since many embryos were noted which were not infected. The external changes induced by the fungus are alone sufficient to induce germination under these conditions.

TABLE IV

Cattleya HYBRID SEEDS INOCULATED AND PLANTED DECEMBER 21, 1922; NOTES
ON RA 28 AND RA 30 MADE JANUARY 2, AND ON RA 25 AND RA 31 MADE
JANUARY 29, 1923

Culture solution and condition	Culture no.	Percentage killed	Average diameter (μ)	P _H
B+2 per cent sucrose	RA 28	0	207	5.6
B+2 per cent sucrose, inoculated....	RA 30	12	225	4.2
B+2 per cent sucrose	RA 25	4	316	5.6
B+2 per cent sucrose, inoculated....	RA 31	37	486	4.2

EXPERIMENT 4.—What will be the effect of the fungus on germination when sugar is supplied in place of starch? Experiments, reported previously, demonstrated that germination is possible with sugar, and according to BERNARD and others the sugar would be a substitute for the fungus. One might expect, therefore, that in cultures containing sugar the fungus would be without any accelerative effect, since a substitute is provided. Or one might expect that if the fungus is of value, due to inducing internal changes, the fungus would thereby favorably influence the embryos. There is finally the possibility that the fungus might accelerate or retard germination because of changing the chemical reaction of the medium.

The cultures were inoculated on the same day that the seeds were planted, and the cultures were kept in the laboratory for three days previous to being transferred to the greenhouse. Within the first two weeks the fungus had accelerated the rate of growth, and

by January 21, one month after sowing, those embryos with the fungus were much superior to those without the fungus.

The embryos of RA 31 (inoculated) were dark green and the leaf point was well defined. The embryos in RA 25, however, were light green and the leaf point was not apparent in any of the embryos. The fact to be noted from the data is that the fungus markedly accelerated germination. The favorable influence of the fungus is here explainable by the change in hydrogen ion concentration of the culture medium. The inoculated cultures had a P_H of 4.2, those uninoculated 5.6. The difference in color and greater growth may be attributed entirely to the more favorable hydrogen ion concentration. This fact has been well demonstrated by experiments made in this laboratory but not yet published.

Seedlings from RA 28, RA 30, and RA 31 were prepared for microscopical examination. RA 28 not being inoculated was of course free from infection; RA 30 and RA 31 showed some embryos completely invaded. Some were but slightly invaded, and others were not infected.

EXPERIMENT 5.—This experiment was essentially like experiment 4, but KH_2PO_4 was used in the culture medium and the seeds were of a different hybrid. The experiment was made at the same time as experiment 3. The data are given in table V.

On October 3 additional notes were made. The inoculated sucrose cultures were superior in size to the uninoculated, and likewise were of a deeper green. Due to the crowding of seedlings, growth had been restricted, and the difference apparent on October 3 would undoubtedly have been greater had more favorable growth conditions been provided. In again looking for an explanation of the favorable influence of the fungus on germination in the sucrose cultures, hydrogen ion and reducing sugar determinations were made on May 15. Solution B+2 per cent sucrose not inoculated had a hydrogen ion concentration represented by P_H 5.6, and the amount of reducing sugar in the culture medium was 15.2 mg. In the inoculated culture the hydrogen ion value was P_H 4.0, while the amount of reducing sugar was 141.2 mg. The amount of culture medium used was 8 cc. to each tube, which theoretically (deducting for 10 per cent moisture in the cane sugar) should have contained approximately 144 mg.

sucrose. The facts of significance here are the change of sucrose to hexose sugars by the fungus, and the change in hydrogen ion concentration. From previous experiments not yet published I am inclined to believe that the inversion of cane sugar is of no particular significance, but the shift in the hydrogen ion concentration is of great importance.

Embryos and seedlings taken from these cultures were prepared for examination. In four or five embryos out of nearly forty examined from culture SA 12, infected cells were noted, but most of the embryos were entirely free of any infection. In the inoculated cultures SA 17, SA 18, and SA 23 not a single embryo or seedling

TABLE V

Cattleya HYBRID SEEDS PLANTED MARCH 15, 1923; CULTURE SOLUTION B+2
PER CENT SUCROSE

DATE OF INOCULATION	CUL- TURE NO.	AVERAGE DIAMETER OF EMBRYOS (μ)				OBSERVATION MADE JUNE 15
		April 12	Culture no.	May 16	Culture no.	
Not inoculated.	SA 7	234	SA 9	387	SA 8	Light green; 5 per cent germinated; 5 per cent dead
March 22.	SA 12	261	SA 17	621	SA 18	Dark green; 90 per cent germinated
April 27.	SA 23	Dark green; 90 per cent germinated

was found to be infected, showing that the action of the fungus is not within the embryo but in the culture medium.

EXPERIMENT 6.—In this experiment another *Cattleya* hybrid was used. The results were like those of experiment 5. There was again a marked increase in growth in the inoculated over the uninoculated culture. The cause of this increase is due entirely to the increase in hydrogen ion concentration. None of the embryos or seedlings when examined in prepared slides showed any infection. Apparently these embryos, as well as those in the preceding experiment, gained immunity as a result of having available a supply of utilizable sugar. Other experiments of similar character were made with like results. The data are given in table VI.

EXPERIMENT 7.—If the action of the fungus is merely on the nutrient solution and without any special value within the embryo,

then a nutrient solution having the same concentration of sugar and the same hydrogen ion concentration as an inoculated culture should accelerate germination to the same degree. That such is the case is apparent from the results of the following experiment. In one series solution B containing approximately 0.05 per cent glucose was used, while the second series contained solution B+0.25 per cent starch.

TABLE VI
Cattleya HYBRID SEEDS INOCULATED AND SOWN FEBRUARY 20; NOTES
TAKEN MARCH 20, 1923

Condition of culture solution	Culture no.	Percentage killed	Average diameter (μ)	P _H
Inoculated.....	DEL I	8	604	4.0
Not inoculated.....	DE I	5	307	5.6

The cultures of the starch series were inoculated on March 22. The seeds were sown on March 15. The data are recorded in table VII. Here the interesting fact is to be observed that the inoculated starch culture having the same hydrogen ion concentration and approximately the same quantity of sugar made almost identically the same growth in the same period of time as was found in the

TABLE VII

Culture solution	Average diameter of embryo (μ)	P _H of culture medium	Glucose in culture (mg.)
Solution B+0.05 per cent glucose.....	239	4.0	3.2
Solution B+0.25 per cent starch inoculated.....	246	4.0	3.7

glucose culture. Certainly the infection of the embryo was of no special value in this case.

EXPERIMENT 8.—In all the preceding experiments the culture medium contained either starch or sugar. The objection might be made that such a medium is not at all comparable with the substrate on which orchids grow. Such an objection may be granted, but the experimental basis for the symbiotic view is based entirely on the use of such media in culture work. In order to answer such criticism, however, an experiment was made in which a mixture of equal parts

of *Osmunda* fiber and *Sphagnum* was used, such as is often employed in the germination of orchid seeds. Thirty-seven gm. of such a mixture was placed in each of eight Erlenmeyer flasks of 500 cc. capacity. To each was then added 110 cc. of solution B, adjusted to a hydrogen ion concentration of P_H 4.6 by the addition of hydrochloric acid. This solution contained neither sugar nor starch. These flasks were then plugged with cotton and sterilized for thirty minutes at 15 pounds pressure. Six of these flasks were then inoculated on May 20 with the orchid fungus and incubated at 26° C. On June 9, by which time the fungus had grown throughout the upper half of the material, two of the flasks were re-sterilized to kill

TABLE VIII

Cattleya HYBRID SEEDS SOWN JUNE 4; NOTES OCTOBER 4, 1923

Treatment	Culture no.	Average diameter (μ)	P_H of solution	Total reducing sugar present (mg.)
Control, no fungus.....	SE 1	495	4.4	18.2
Control, no fungus.....	SE 2	531	4.4	480.0*
Inoculated May 20.....	SE 4	531	4.4	40.0
Inoculated May 2.....	SE 6	531	4.4	10.8
Inoculated May 20.....	SE 8	378	4.4	40.0

* Contaminated with *Penicillium* sp.

the fungus. There remained two flasks which had not been inoculated and which were the controls. On June 10, seeds of a *Cattleya* hybrid were planted in all the flasks, and these were then placed in the greenhouse. Observations were made on all these flasks on October 4, 1923. The data are given in table VIII.

The hydrogen ion concentration was determined by extracting 5 gm. of the medium with 10 cc. of distilled water. This was then centrifuged and the supernatant liquid used for the determination. Sugar was only roughly determined by extracting the entire mass for 24 hours with 95 per cent alcohol. The whole mass was then thrown on to filter paper and the alcohol drained off. This mixture was then washed with alcohol, and the alcoholic filtrate placed on a water bath. When dry, the residue was redissolved in 100 cc. of distilled water, filtered, cleared by means of neutral lead acetate, and the lead removed by means of sodium sulphate. The filtrate

obtained was then analyzed for reducing sugar. The high hydrogen ion concentration prevailing at all times rendered it unnecessary to examine for sucrose.

In cultures SE 1, 2, and 6 various seedlings were noted having one leaf, and in others the leaf was just appearing. In culture SE 8 only one seedling with a leaf was noted. Cultures SE 3, 5, and 7 became contaminated with a *Penicillium*, but a few seeds near the walls of the flasks which were not covered by the fungus made excellent development, being superior to any of the others.

It is obvious from table IX that the inoculated cultures were no better than those of the control cultures which lacked the fungus. SE 2 was contaminated with *Penicillium* sp., which accounts for the high content of sugar. The growth of the fungus was markedly less in the SE 6, which may account for the less sugar. The fact of greatest significance is that germination occurred in the flask lacking any fungus. If the orchid fungus is essential, we should not expect germination under these conditions.

The objection might be made that the material was sterilized; but certainly the concentration of sugar was low, only 18.2 mg. in about 100 cc. of culture solution. Again, objection might be made on the basis that solution B was added, and that the hydrogen ion concentration was adjusted; but, with the same hydrogen ion concentration, germination has not been obtained on solution B in any agar cultures. The evidence points again to the conclusion that germination of orchid seed is absolutely dependent on soluble organic food obtained from external sources.

EXPERIMENT 9.—This experiment deals with the effect of various fungi on germination of orchid seeds. It has been shown in the preceding experiments that the orchid fungus will induce germination when the seeds are planted on medium containing starch, and likewise that the germination of seeds is accelerated when sugar is supplied. The explanation offered is the change in hydrogen ion concentration in the sucrose cultures, and in the starch cultures the increase in hydrogen ion concentration combined with the formation of sugar. In the light of these facts it may confidently be expected that any fungus which can digest starch, changing it to sugar, and likewise increase the hydrogen ion concentration, should be capable

of inducing germination, provided that the growth of the organism is not great enough to smother the seed, or provided that the fungus is not capable of infecting and killing the embryos.

TABLE IX

EFFECT OF VARIOUS COMMON FUNGI ON GERMINATION OF *Cattleya* HYBRID;
SEEDS SOWN APRIL 14; NOTES MADE AUGUST 19, 1923

Series	Fungus	Date of inoculation	Condition
A.....	Phytophthora sp.	6-15-23	All very good; none dead; dark green and healthy looking; decidedly better than checks
B.....	Sclerotinia libertiana	6-15-23	All plants dead and brown
C.....	Oidium lactis	6-15-23	Fungus failed to grow; no better than checks
D.....	Sclerotinium sp.	6-15-23	All plants dead and brown
E.....	Choanophora cucurbitarum	6-15-23	Plants still green and healthy looking; not as good as A but better than checks
F.....	Penicillium camembertii	6-15-23	Plants in four of tubes green and healthy but developing slowly; in fifth tube three well developed fern prothallia present, apparently as contamination of orchid seed; in this tube orchids growing much more rapidly, darker green, and looking slightly better than those in series A
G.....	Corticium vagum	6-15-23	Good development at first but now turning brown; two of tubes have fern prothallia in them and here seeds appear better than in remainder
H.....	Mortierella rhizogena	6-15-23	Seed is practically all smothered by dense growth of fungus; those still uncovered look better than checks
I.....	Cucurbitaria sp.	6-15-23	Seeds still healthy; dark green and developing much better than checks
J.....	Cladosporium citri	6-15-23	Now becoming smothered by fungus; those not covered are of good green color and better than checks; one tube has two fern prothallia
K.....	o-22 (orchid fungus)	7-19-23	Much better than checks; dark green and developing rapidly; about equal to series A
L.....	No fungus	6-15-23	Seed still green but very small; decidedly inferior to those in series A, E, F, I, and J

Solution B + 0.5 per cent soluble starch was used and 10 cc. was added to each tube. The tubes were then plugged, sterilized, and sloped. Some days after planting, the tubes were inoculated, and notes taken, as indicated in table IX. It may be noted that in many cases where the seeds were not covered or killed by the fungus, the embryos were more advanced than in the control cultures. The

cultures inoculated with *Phytophthora* sp. were especially favorable to growth of the embryos. Here we have suggestive evidence of the favorable action of various fungi. Unfortunately, the rate of growth of some of the fungi was too great, and most of the seeds were covered by the developing mycelium.

Further observations were made on these cultures on October 27, and the data are given in table X. At this time hydrogen ion determinations and reducing sugar determinations were also made.

TABLE X

Cattleya HYBRID SEEDS PLANTED APRIL 14; NOTES TAKEN OCTOBER 27, 1923

Series	Fungus used	Average width of embryos	Percent age with leaves	P _H of solution	Reducing sugar present	Notes
A.....	<i>Phytophthora</i> sp.	432	11	5.0
A.....	<i>Phytophthora</i> sp.	369	5	5.1	13.5	Starch in tube entirely digested
A.....	<i>Phytophthora</i> sp.	423	10	5.0	22.8	Starch in tube entirely digested
K.....	Orchid fungus	468	10	4.6	Starch in tube entirely digested
K.....	Orchid fungus	477	16	4.5	16.9	Starch in tube entirely digested
K.....	Orchid fungus	468	11	4.5	11.1	Starch in tube entirely digested
E.....	<i>Choanophora cucurbitarum</i>	288	4*	5.4	0.0	Starch in tube entirely digested
G.....	<i>Corticium vagum</i>	261	0†	6.4	1.8	Starch in tube entirely digested
L.....	No fungus	261	0	6.4	1.8	Starch abundant in tube
L.....	No fungus	243	0	6.4	1.4	Starch abundant in tube
L.....	No fungus	225	0	6.4	Starch abundant in tube

* Seeds germinated only where not covered by fungus.

† Seeds smothered by fungus; in one tube two seedlings were produced somewhat later.

Table X shows that the *Phytophthora* sp. (isolated from Easter lily) is about as effective as the orchid fungus in inducing germination. There is a slight difference in the size of the embryos, but this may be attributed to the lower hydrogen ion concentration. With *Choanophora* a few seedlings were produced. These were just above the mycelial mat. This organism grew more vigorously than either the orchid fungus or *Phytophthora*, which probably accounts for the complete absence of sugar in the culture medium, although the starch had been entirely digested. With *Corticium vagum* the starch was entirely digested, little sugar was present, but the hydrogen ion

concentration (P_H 6.4) was unfavorable to growth. The embryos likewise were enmeshed within the ramifying hyphae. The fungi that induced germination produced comparable changes in the culture medium, namely, increase in the hydrogen ion concentration and digestion of starch with the production of sugar. The concentration of sugar in series K at the conclusion of the experiment (8 cc. remaining of the original 10 cc.) ranged from 0.138 to nearly 0.21 per cent, while in the *Phytophthora* series the concentration ranged from 0.168 to 0.285 per cent sugar. In cultures with *Penicillium camembertii*, and also in the cultures with *Mortierella rhizogena* a few seedlings were produced near the upper portion of the slope and above the fungus mat. Most of the embryos were either retarded or

TABLE XI

Cattleya HYBRID SEEDS PLANTED FEBRUARY; NOTES MADE JUNE, 1923

Culture conditions	Culture no.	Average diameter (μ)
Solution B+ $\frac{1}{2}$ starch, not inoculated.....	SC 2	225
Solution B+ $\frac{1}{2}$ starch, inoculated seed on surface of medium..	SC 32	432
Solution B+ $\frac{1}{2}$ starch, inoculated but seed adhering to wall of tube and not in contact with agar.....	SC 32	198

killed when covered by the fungus. Such data permit of the conclusion that the external changes only are important.

Prepared slides were made of a large number of seedlings and embryos taken from all of the series included in table XI. These preparations were carefully made and thoroughly examined, not only with the usual dry objectives, but also at magnifications of 1200. In no case was any embryo or seedling infected. The strikingly beneficial effect of *Phytophthora* and the orchid fungus, therefore, could not have been due to any internal action of the fungus. I did not expect to find infection with *Phytophthora* sp., but I had expected to find it with the orchid fungus. These cultures of series K were inoculated three months after the seeds were sown, however, and apparently the embryos had become entirely immune to the fungus.

EXPERIMENT 10.—BURGEFF, in describing one of his experiments with the fungus, stated that the embryos adhering to the inner

surface of the tube out of contact with the culture medium germinated. I suggested that possibly the embryos were in contact with droplets of the agar medium adhering to the surface, and that the products of digestion or excretion were adequate to account for growth. There was also the possibility that, in examining the tube, seedlings accidentally became detached from the slope and were flipped to the side of the tube. If such embryos on the side of the tube could germinate when out of contact with the culture medium, then we would have rather strong evidence in favor of the fungus hypothesis.

That embryos adhering to the tube wall out of contact with agar do not germinate, and are no better than the embryos on starch media uninoculated, has been noted many times in the course of my experiments. In at least eight different inoculated cultures with solution B + $\frac{1}{4}$ starch, seeds have been noted adhering to the wall of the tube, and no stimulating effect of the fungus was noted. In table XI, data are given on the growth of seeds on the walls and on the agar surface of an inoculated starch medium. For comparison, there are given the growth measurements for the uninoculated starch culture. The embryos adhering to the wall were all infected, so that lack of infection was not responsible for any failure to germinate. They were likewise of the usual green color, but not having available sugar and possibly also organic acids produced by the fungus, germination was not possible.

EXPERIMENT II.—That the effect of the fungus on orchid seed germination is external and not internal is further shown by the following experiment. Six series of cultures were prepared with solution B and various concentrations of starch. The inoculated series had starch contents of $\frac{1}{4}$, $\frac{1}{2}$, 1, and 2 per cent respectively. The control cultures consisted of one series with $\frac{1}{4}$ per cent starch and a second with 2 per cent starch. The treatment of these two series was identical with the preceding, except that they were not inoculated. All of these cultures were planted with the same seeds and maintained under the same conditions. On January 10 measurements were made of the embryos, the figures given under average width being again the average of fifty measurements. Hydrogen ion concentration and reducing sugar were also determined. The data

are given in table XII. It may be noted that, in the two control cultures with $\frac{1}{4}$ and 2 per cent starch, not inoculated, the average size of the embryos is only 124 μ . In the inoculated cultures there is an increase in growth. The higher the concentration of starch the greater the sugar content, and therefore the greater the growth. It may be noted also that the hydrogen ion concentration of the inoculated cultures was increased in all cases to P_H 4.7 by the fungus.

These data give emphasis again to the external changes as the important factor in germination under pure culture conditions. Certainly if the effect of the fungus were within the embryo, we should not expect differences of the character observed.

TABLE XII

Cattleya HYBRID SEEDS PLANTED NOVEMBER 10, 1923; NOTES MADE
JANUARY 10, 1924

Culture solution	Inoculated	Culture no.	Average width of embryos (μ)	Maximum width of embryos (μ)	P_H of culture medium	Glucose per culture tube on January 10 (mgr.)
Solution B + $\frac{1}{4}$ per cent starch..	December 11	SL 2	211	291	4.7	5.9
Solution B + $\frac{1}{2}$ per cent starch..	Not inoculated	SM 7	124	144	5.5	0.0
Solution B + $\frac{3}{4}$ per cent starch..	December 11	SM 1	268	384	4.7	28.7
Solution B + 1 per cent starch..	December 11	SN 1	311	480	4.7	63.8
Solution B + 2 per cent starch..	December 11	SO 2	393	576	4.7	121.8
Solution B + 2 per cent starch..	Not inoculated	SO 7	124	144	5.5	0.0

EXPERIMENT 12.—Some of the previous experiments indicated that there is a relation between the physiological state of the embryos and the degree of infection. This was shown in experiment 1, as well as in other experiments. Some of the data suggested that the longer the interval of time between the date of sowing and the date of inoculation, the less would be the destructive action of the fungus.

In order to determine specifically whether or not the interval of time between the date of sowing and the date of inoculation is a factor to be considered, the following series of experiments was started. Eighty tubes were prepared with solution B, using KH_2PO_4 instead of K_2HPO_4 , and $\frac{1}{4}$ per cent starch. Beginning on October 24, ten tubes were planted with seeds of a *Cattleya* hybrid. At in-

tervals of every two days until November 9, ten more tubes were sown with the same seeds, so that the last seeds were sown sixteen days later than the first. Then on November 9 five tubes of each series were inoculated with the same culture of the orchid fungus. By this procedure some cultures were inoculated sixteen days after the seeds were sown, others fourteen days later, others twelve days after sowing, and so on, until one lot of cultures was inoculated on the same day that the seeds were planted.

At intervals of time the cultures were examined, and finally on April 11 notes were taken on these cultures. In the uninoculated cultures the embryos were in the small spherule stage, being approximately $220\ \mu$ in diameter. They were green and still living. In the inoculated cultures the results were all alike. Of approximately 200 seeds planted in each tube, only one or two germinated. The remainder were white or brownish, varying in diameter from 180 to $450\ \mu$. The smaller embryos were heavily infected, and masses of hyphae radiated from the embryos. The larger embryos were also heavily infected and the cells appeared to be disorganized, showing the pathogenic character of the fungus.

That the activity or the capacity of the fungus was not altered by being kept under pure culture conditions is evident from the following experiment. Ten of the original control cultures (uninoculated) were inoculated with a subculture of the same organism on April 11, 1924. Others of these control cultures were inoculated with the fungus from *Epipactis* and *Cypripedium*. On May 10, most of the embryos were just producing the leaf point and only a few were killed. By May 22 most of the seeds had germinated. The embryos in cultures of the previous experiment inoculated sixteen days after planting were practically all killed, but the embryos of this lot inoculated six months later with the identical fungus, or inoculated with the fungus from *Epipactis* or *Cypripedium*, were capable of germination, only a few of them being killed. This resistance on the part of these embryos is owing to physiological or cell wall changes due to prolonged culture, or else the embryos become resistant because of a higher starch content of the medium, and consequently on digestion of starch by the fungus a higher sugar concentration.

When the tubes were prepared the concentration of starch was 0.25 per cent, but as a result of evaporation the uninoculated tubes had a starch concentration of approximately 0.5 per cent on April 11. Previous experiments with media containing sugar or high starch content, which when inoculated is equivalent to high sugar content, showed that the embryos gain immunity by being supplied with a relatively high concentration of sugar. This aspect of the subject is being investigated at the present time.

EXPERIMENT 13.—One of the strongest arguments advanced in favor of the symbiotic view is that the fungus from one genus of orchids may be ineffective in causing germination of seeds of orchids of other genera. While the fungus from *Cattleya* is effective for the seeds of *Cypripedium*, *Cymbidium*, and *Epipactis*, it is claimed to be without effect on the germination of seeds of *Odontoglossum*. Likewise the fungus of *Odontoglossum* does not bring about germination of *Cattleya* seeds. Lacking plants of *Odontoglossum*, I have not as yet been able to obtain this fungus, but the effect of fungi isolated from *Cattleya*, *Epipactis*, and *Cypripedium* on seeds of *Odontoglossum* was determined.

For determining the effect of these fungi, five tubes of solution B + $\frac{1}{4}$ starch were sown with seed of an *Odontoglossum* hybrid, and inoculated with the fungus from *Cattleya*. This was repeated with the fungus from *Epipactis* and *Cypripedium*. Another similar series was provided, using $\frac{1}{2}$ per cent starch in place of $\frac{1}{4}$ per cent. With these fungi there was no germination. Upon examination it was found that every seed had been killed. Most of the seeds were completely invaded, and masses of hyphae were present. The failure of the fungi isolated from *Cattleya*, *Cypripedium*, and *Epipactis* to produce germination is due, not to failure to infect the seeds, but to the extremely pathogenic character of the fungus for the seeds of *Odontoglossum*. Seeds of *Odontoglossum* sown on solution B + 2 per cent sucrose adjusted to P_H 4.5 germinated and made excellent growth.

Summary of experintents

1. A fungus resembling morphologically that described by BERNARD as *Rhizoctonia repens* was isolated from *Cattleya*, *Cypripedium*, and *Epipactis*.

2. This fungus was capable of inducing seeds of *Cattleya* to germinate on a medium containing starch.

3. This fungus accelerates the growth of orchid seeds in solution B containing sucrose.

4. The fungus may permit of complete germination or may kill every seed in the culture. One of the factors which controls the degree of infection is the concentration of starch used. This means, of course, that ultimately it is the concentration of sugar.

5. In the culture medium containing starch in which germination occurs when the fungus is present, the following changes occur. The starch finally is completely digested, and sugar is produced. The hydrogen ion concentration is changed from a value which is unfavorable for growth, to one which is favorable. These external changes are sufficient to bring about germination.

6. Germination may be effected by the fungus, even though no seeds are infected.

7. The fungus hastens germination of seeds in solution B+2 per cent sucrose. The acceleration is due to the increase in hydrogen ion concentration, and not to any internal action, for the embryos are not infected.

8. Germination was obtained without the fungus on a peat and sphagnum mixture with solution B adjusted to P_H 4.6, and the germination was just as rapid as when the fungus was supplied. The fungus is without any value here.

9. The growth of the embryos with solution B+0.05 per cent glucose, with a hydrogen ion concentration P_H 4.6, is about the same as with solution B+ $\frac{1}{4}$ per cent starch inoculated with the fungus. The sugar content and hydrogen ion concentration of the latter were practically the same as those of the glucose culture.

10. *Phytophthora* sp. is about as favorable to germination as the orchid fungus. The chemical changes induced in the culture medium by the former are nearly the same as those produced by the orchid fungus. Germination was effected by other fungi.

11. In an experiment in which solution B was used with concentrations of starch varying from $\frac{1}{4}$ to 2 per cent, with the fungus, the growth increased with the concentration of starch. With $\frac{1}{4}$ per cent starch, germination was not attained, even after six months. For

each increase in starch content there resulted, because of digestion by the fungus, a progressive increase of sugar. The hydrogen ion content was the same in all cases.

12. In determining the effect of the fungi isolated from *Cattleya*, *Epipactis*, and *Cypripedium* on seeds of *Odontoglossum*, it was found that these fungi are extremely pathogenic, and practically every seed was killed. Without the fungus the seeds turned green and were still living after six months; with sugar supplied, the seeds germinated.

Discussion

The symbiotic theory rests very largely upon two facts. The first is that the roots of orchids are generally infected by a fungus, which fungus is generally believed to be of some value to the orchid. At present, however, it must be granted that no evidence has yet been presented which indicates any favorable effect of this infection on the orchid plant. The presence of the fungus in the roots of orchids is not evidence that the fungus is essential. The association of fungus and root may be merely incidental and not of any significance. CONSTANTIN and MAGROU go so far as to state that the relationship is quite intimate, for they practically state that it is no more permissible to consider an orchid, which lacks the fungus, as an orchid, than it is permissible to consider the individual plant components of a lichen as a lichen. The fact remains, however, that no proof has ever been presented to show any beneficial effect of the fungus, unless we accept the conclusions of KUSANO (9), who stated that *Gastrodea elata*, a rather unusual Japanese orchid, will not flower unless infected by *Armillaria mellea*. Incidentally this organism is quite distinct from the real orchid endophyte.

The proponents of the symbiotic view state that the experiments of BERNARD and BURGEFF prove that the orchid fungus is essential for germination. In my first paper I stated that under the conditions of pure culture, as followed by BERNARD and BURGEFF, the fungus was essential, but I suggested that the fungus was of value in these cases only because it changed starch to sugar or increased the quantity of other soluble organic food as a result of digestion processes and by excretion of favorable substances. The experiments reported in this paper show that the external changes induced by

the fungus are sufficient to explain germination when the fungus is supplied. These changes induced by the fungus are digestion of starch, formation of sugar, and the production of a favorable hydrogen ion concentration. These changes are in themselves adequate for inducing germination. In addition to these facts, however, evidence is presented showing that even on a starch medium, infection of the seedlings did not always occur, although the seeds germinated. Germination on a starch-containing medium was obtained also with fungi not related to the orchid fungus. To be sure this fungus was slightly superior, but the more favorable action of this fungus could be explained on the basis of more desirable changes induced in the culture medium.

BERNARD emphasized the fact that a delicate balance had to be maintained between the fungus and embryo. He states: "The germination by inoculation is not without certain difficulties. . . . For the majority of seeds, the association with the fungus that I have placed in their presence has been merely passive and without effect, or impossible or rapidly injurious to the embryos."

CONSTANTIN and MAGROU have criticized me for quoting BERNARD in this manner. This quotation was made to lend emphasis to the idea that the fungus was at times a real pathogen. I stated in my first paper as follows: "It is possible that the fungus instead of being an aid in normal germination, is a factor in the death of the embryos and consequently in the failure of germination." That such is the case must be apparent from data supplied in experiment 12, where less than 1 per cent of the seeds were able to germinate after a period of six months. In the control cultures not inoculated the seeds were still all alive.

One of the arguments that might be offered in favor of the symbiotic conception is from the experiments of BERNARD on the difference in "activity" of the fungus when isolated from different species of orchid, and also the so-called loss in activity when the fungus is grown under pure culture conditions. If the interpretation put upon these experiments by BERNARD were accepted, it would be necessary to conclude that symbiosis plays a rôle in orchid seed germination, but I am convinced that this interpretation is not correct. Before considering in detail one of these experiments, it is necessary to

define what BERNARD means by activity. He states: "I have considered that fungus the most active which, all other conditions being equal, causes the most rapid germination, the greatest number of seedlings, or the best developed seedlings in a given time." In this particular experiment BERNARD determined the influence of the duration of pure culture conditions on the activity of the fungus. The initial isolation of the fungus was made December 1905, and, of course, repeatedly transferred during the subsequent three years, when finally the activity of the fungus was compared in its influence on germination with the activity of subcultures of this same fungus. The different subcultures during the three years, however, had

TABLE XIII

Culture designation	Date of isolation	Origin	Subsequent treatment	Pure culture
Mycelium C..	December, 1905	3 years
Mycelium C ₁ ..	June 6, 1905	C	March 1 to June 6, 1906, in Laelio seedlings (67 days)	19 months
Mycelium C ₂ ..	October 26, 1906	C ₁	June 15 to October 26, 1906, in Laelio seedlings	14 months
Mycelium C ₃ ..	May 3, 1907	C ₂	November 14, 1906, to May 3, 1907, in <i>Cattleya</i> seedling	8 months
Mycelium C ₄ ..	November 14, 1907	C ₃	July 1 to November 14, 1907, in <i>Cypripedium</i> seedling	1.5 months

each a different history. The fungus kept under pure culture conditions throughout the period was labelled C, but C₁ had the following history. It was used for inoculating seedlings of *Laelio* on March 1, 1905, and reisolated on June 6, 1905. Thereafter it was maintained under pure culture conditions. The history of the other subcultures is indicated in table XIII. The results were striking. In the tube inoculated with culture C, the seeds did not exhibit any development whatsoever; with culture C₁ only a single seedling was produced, with but a very small percentage of seeds which showed any appreciable development; with C₃ approximately 5 per cent of the seeds produced seedlings; while with C₄ a high percentage of seedlings was produced. The data indicated that the shorter the time between the date of isolation and the date when the fungus is used for inoculation, the more effective is the fungus in inducing germination.

Assuming that the orchid cultures were all maintained under

identical conditions, one would have to conclude that the difference in the life histories of the fungus is a factor in its ability to induce germination. This may be accepted, but the explanation is not a loss of activity in the sense that the seeds are not injured, but the failure of germination is due to the fact that the fungus has behaved as a strong pathogen, and with cultures C and C₁ most of the seeds were killed by the fungus. In other words, the most active fungus is the one which is the weakest pathogen.

In the course of my various experiments with the fungus I have had germination varying from 0 to 100 per cent. I have not as yet had the opportunity to determine precisely what conditions make for a low or a high percentage of germination. In general, however, the results indicate that the higher the starch content of the culture medium, the higher will be the sugar content of the medium, and consequently the lower the mortality. The composition of the nutrient solution, the length of the interval between the date of sowing and the date of inoculation, and the kind of seeds used are all factors to be considered. Some preliminary experiments indicate also that the composition of the medium on which the fungus has grown is a factor in the virulence of the organism.

Another argument advanced by RAMSBOTTOM in favor of the symbiotic conception is that the fungus of *Odontoglossum* is without any effect on *Cattleya*, and likewise the *Cattleya* fungus is without any effect on *Odontoglossum*. I have tested the fungi isolated from *Cattleya*, *Epipactis*, and *Cypripedium* on *Odontoglossum* seed, and in each case the seeds were killed. The significant fact is that the fungi from *Cattleya*, *Cypripedium*, and *Epipactis* all killed seeds of *Odontoglossum*. This being so, it is possible that one of the difficulties in the germination of *Odontoglossum* seeds is that the fungus from *Cattleya* is present in the greenhouse, and is particularly destructive to the embryos of *Odontoglossum*.

Other evidence that might be cited from the work of BERNARD in favor of the symbiotic view of orchid seed germination is his experiments with *Bletilla hyacintha*. He found it possible to germinate seeds of this orchid without the use of any fungus, but the seedlings produced were somewhat attenuated. With the fungus the seedling formed a protocorm and was more compact in structure.

BERNARD maintained that the fungus, through internal action, induced chemical changes resulting in a high concentration of sap. The high concentration of sap in turn caused the difference in the type of seedling. BERNARD's evidence that the fungus causes an increase in concentration of the cell sap was that he grew the fungus in a nutrient solution containing salep and sucrose, and found that it produced an increase in concentration of the nutrient solution, this increase being due largely to the inversion of sucrose and digestion of starch. Here was a clue to a rational explanation of the function of the fungus in his experiments, but, from the fact that the fungus brings about an increase in concentration of the nutrient solution, he assumed that the same may take place in the embryo.

In interpreting the differences in the structure of *Bletilla* seedlings with and without the fungus, one must take into consideration a number of facts. In the first place, any plant growing in a small inclosed tube or flask which is tightly stopped with cotton will be restricted in growth due to a decreased rate of photosynthesis. In another paper (8) I presented evidence to show that the cotton stopper impeded the diffusion of carbon dioxide into the vessel. MOLLARD (10) demonstrated that radish plants growing in flasks under pure culture conditions produced enlarged roots only when a relatively high concentration of sugar was provided. BERNARD explained the formation of storage roots on the basis of concentration, but the logical explanation is that the storage root is formed only when sufficient food is present.

The compact type of seedling formed by *Bletilla* when the fungus is present is explainable then on the fact that the fungus changes insoluble foods in the salep to soluble foods, and that as a result of respiration of the fungus the carbon dioxide content of the tube is augmented over that in tubes without the fungus.

In considering finally the evidence for the necessity of the fungus for germination, and, as CONSTANTIN and MAGROU state, the necessity of the fungus for the normal development of the plant, it must be granted that the only evidence is the almost constant association of fungus and the orchid plant. That the fungus is necessary for growth of the orchid is not true. In my second paper I described an experiment in which seed of *Cattleya* were germinated without the

fungus, but with sugar. When the embryos were about 0.6 mm. in diameter, they were transplanted to a nutrient solution entirely lacking in any organic food. These seedlings were transplanted several times. The largest seedling now, after three years of growth, has five large leaves 8-15 cm. in length, and well formed pseudo bulbs. The plant, except for the first two months, has been supplied merely with solution B, and has been grown entirely within a flask. Any orchid grower would pronounce it a very good three-year-old plant.

The explanation of the failure of orchid seeds to germinate when provided with all the conditions that permit of the germination of most seeds, is to be found in the organic food relations. The seeds of orchids are lacking in food reserves. There is no endosperm or cotyledon, but the seed is a simple undifferentiated embryo. I have made various analyses of seeds of *Cattleya* for sugar, starch, and fat (ether extract). No starch was present; the total sugar amounted to 1.2 per cent; while the fat content was equal to 32 per cent of the dry weight. Growth of the embryo will continue for a time at the expense of the reserve food, but ceases sooner or later. If the embryos are then supplied with sugar, growth will continue and germination occur. As presented in my second paper, this suggests that orchid embryos are dependent on an outside source of organic food for continued development. This must signify that even in those embryos that are provided with chlorophyll, photosynthesis is lacking. It would seem that photosynthetic activity in the orchid is delayed, due to the lack of some internal factor, as reported by BRIGGS (2, 3) for other seeds. If the embryos are carried over this critical period, then they are thereafter self-sustaining.

The significant fact has been noted that the seeds of terrestrial orchids may germinate in nature under conditions where chlorophyll is entirely lacking. These embryos are purely saprophytic. The conclusion seems to be warranted that under natural conditions the orchid embryos are dependent for continued development on an appropriate supply of organic food, which must be absorbed from the material on which the seeds are germinating. Under natural conditions this food is made available to the orchid embryo by the digestion of organic matter, which transforms the insoluble sub-

stances to soluble products. Some of these substances are absorbed by the embryos and are used in the metabolic processes. Under natural conditions the orchid fungus may function in these digestive processes, but it would be pure assumption to conclude that no other microorganisms are involved in this transformation. Sugars are undoubtedly formed, although the concentration would be low. It would seem that other substances are more effective. Additional discussion of this aspect of the problem may well await further investigations on the photosynthetic and food relationships of orchid embryos.

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TROPICAL LIGHT WEIGHT WOODS

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(WITH PLATES XXVI-XXVIII)

Light weight woods have come into prominence in the last decade, as the source of material for the manufacture of "buoyant and insulating products." The extent (15) to which the wood of *Ochroma* was utilized by the allied nations during the recent war, in the production of lifeboats, refrigerators, floats, and in the construction of parts for aeroplanes is well known. CARPENTER (4) studied the wood of *O. lagopus*, and has briefly set forth its anatomical and physical characteristics. He has correlated these characteristics with the efficiency of the products, by showing that the insulating properties of this wood are much higher than those of any other substance in commercial use, this being due to its peculiar histological structure. The properties of its high insulation against heat, its light weight, and proportionately great structural strength, are no doubt its most valuable qualities (1). The utilization of these woods for lining refrigerator cars, for cold storage plants, and for iceboxes has become very general during the past four or five years. This has resulted in decreasing considerably the losses formerly experienced in the marketing of perishable products, and has consequently increased the efficiency of the carriers of foodstuffs throughout the world.

As has been pointed out by ROWLEE (16), light weight woods fall naturally into two categories: (1) those in which the elements are arranged in a relatively homogeneous mass; and (2) those in which soft parenchymatous masses alternate with more or less continuous bands of thick walled elements. The first are homogeneous, the second laminated woods. This latter type of wood occurs frequently in the tropics, some species presenting a more laminated structure than others. VOLKERT (20), in a histological study of *Jacaratia spinosa* (Caricaceae), found that the only lignified elements in the stem are the vessels; that the wood resembles the tissue of a turnip; and upon drying loses its form, and shrinks to a small fraction of its

original volume. The nearest approach to a laminated type of wood in northern latitudes occurs in the Juglandaceae, where very thin tangential bands of parenchyma occur in the annual ring. These are especially prominent in *Hicoria*.

Geographical distribution

Trees producing light weight wood appear to be confined to certain geographical areas, and in general their distribution is limited to the zone shown on SCHIMPER'S (17) map as the "luxuriant tropical rain forest." With the exception of *Adansonia*, which includes the "baobab" tree of Africa, the trees are confined to the wet, hot regions of Central and South America.

In Central America the trees are rarely found in the dry regions of the south coast, northwest of Panama, a region much drier than that along the north coast. In Central Panama the conditions are somewhat different. South and east of the Canal Zone, the humid region is not confined to either coast, but involves the whole breadth of land areas, from coast to coast. The rainfall and humidity gradually become greater on the south and west coasts, whereas the north and east coasts become relatively dry as one approaches Venezuela.

On the west coast of Colombia the humid region continues, especially along the foot of the Andes, as far south as southern Ecuador. This is indicated by the large number of species common to the north coast of Central America, and to the west coast of Colombia and Ecuador. It is in this continuously humid, hot belt, centering in Panama, that these trees reach their maximum both in size and numbers. Here are found species of the following genera that produce light weight woods: *Ochroma*, *Heliocarpus*, *Pachira*, *Apeiba*, *Cordia*, *Cavanillesia*, and *Wercklea*. There are no doubt others that will be discovered with further exploration. The majority of trees producing light weight wood belong to the closely related families Tiliaceae (*Heliocarpus*, *Apeiba*), Malvaceae (*Wercklea*), and Bombacaceae (*Pachira*, *Ochroma*, *Cavanillesia*). To the Boraginaceae, a somewhat unrelated family, belongs *Cordia*.

Material and methods

Because of the increased economic importance of light weight woods and of the almost complete lack of literature concerning them,

the writer undertook a detailed histological study of representatives of all the preceding genera, with the exception of *Ochroma*. For the structural study of laminated wood, four species were used which differ markedly in the degree of lamination, as well as in other histological characters. They are *Heliocarpus popayanensis*, *H. appendiculatus*, *Apeiba aspera*, and *Cordia heterophylla*. For the study of homogeneous light weight wood three species were used, namely *Pachira barrigon*, *Cavanillesia platanifolia*, and *Wercklea insignis*.

The method employed in sectioning the wood, in general was as follows. The areas for treatment were carefully selected to avoid knots, season checks, or other irregularities, and blocks approximately 6 mm. square were cut. Care was exercised to expose perfect transverse, tangential, and radial aspects on the surface of the block (3). The blocks were given a thorough boiling to drive out the air, but in doing this it was necessary to be very careful, as a prolonged boiling caused the tissue to crumble. This process did not remove all the air, and it was necessary to use the air pump for this process. The blocks were then treated with a 50 per cent solution of hydrofluoric acid for four to eight days, depending upon the hardness of the wood. After washing out the acid, the blocks were usually imbedded in celloidin, according to the method given by PLOWMAN (11). In some cases it was found that by softening the blocks for two weeks in a mixture of 50 per cent alcohol and glycerine, after removing the acid, satisfactory sections could be cut on the sliding microtome without previous infiltration. For staining the sections, Delafield's haematoxylin in combination with safranin gave the best results. For examining the sculpture of the walls, unstained sections mounted in glycerine jelly were very satisfactory. For studying the individual elements, macerated material mounted in glycerine jelly was used to advantage. To make the elements more easily studied, they were stained in a saturated solution of nigrosin in picric acid, according to the method given by LEE (8). This helped appreciably in outlining them with the camera lucida.

Heliocarpus popayanensis

LOCALITY AND HABIT OF GROWTH

Heliocarpus belongs to the Tiliaceae. It is exclusively a genus of the American tropics, being found in southern Mexico and extending

from this northern limit to Chili. The number of species in the genus is questionable, but according to ROSE (14) is between fifteen and twenty. *Heliocarpus popayanensis* was first described in 1821. The type specimen was collected near Popayan (6), United States of Colombia. The specimen from which the wood for this study was taken corresponds closely to the type, and was collected between Huigra and Bucay, Ecuador, July 6, 1921, at an altitude of approximately 1000 m.² The plant has been reported from Mexico, Ibaguë, Island of Tobago, Panama, Santa Marta, and Bolivia. The species is sometimes confused with *H. americana*, but differs from it in being a much larger tree, and in having larger and more pronounced trilobed leaves. The tree is called Balsa Menudito in the Magdalena Valley. The natives of Ecuador call it Balsa Bobo, or Fool's Balsa, because of its superficial resemblance to *Ochroma*, the wood of which is known by the Spanish name Balsa throughout Central and South America.

The tree from which this sample was taken was 25 cm. in diameter, and a short distance above the ground divided into several branches. In outward appearance it resembled the American smoke-tree, *Rhus cotinoides* Nutt. It had the general appearance of a plant that was growing out of, or at, the extreme limits of its range. The tree, according to the collector, occurs quite commonly throughout Ecuador. It is closely related to the basswoods of North America.

GROSS ANATOMY

The wood of this tree belongs to the category of a light weight laminated wood. The laminations are very pronounced near the pith, but near the periphery they are scarcely noticeable until sections of the wood are stained. The light weight of the wood is its most striking characteristic. This lightness is due to the thinness and the unligified character of the walls of the bulk of the elements composing the wood, together with the comparatively large, air-filled lumina. The wood is white with a tendency toward flesh color. There is no perceptible difference in the color of the sap wood and that of the heart wood, but the latter is softer, lighter, less lignified, and less fibrous than the former. The wood is odorless and tasteless. Near the periphery it weighs 19 pounds per cubic feet when oven-

² All species discussed in this paper were collected by Professor W. W. ROWLEE, unless otherwise stated.

dried to constant weight at a temperature of 100°C . It has a specific gravity of 0.30. Near the pith it weighs as low as 12 pounds per cubic foot. "Ripple marks" are readily seen with the unaided eye on the surface of the longitudinal cuts. These are more prominent on the tangential than on the radial surface. The height of the markings is fairly constant, and averages 0.38 mm. RECORD (13) has called attention to the presence of "ripple marks" in several other species of the Tiliaceae.

MINUTE ANATOMY

VESSELS.—The vessels as they appear in a transverse section are irregularly distributed throughout the wood (fig. 1). They are more numerous in the lignified than in the unlignified areas. They are mostly round or oval in outline; some of them, however, as a result of crowding in groups, appear to be subdivided radially. The average obtained from fifty measurements of the tangential diameters of the pores was $150\ \mu$, while of the radial diameter, the average of a like number of measurements gave $186\ \mu$. The thickness of the walls varies from 6 to $11\ \mu$, but the average is about $8\ \mu$. The vessels lack tyloses, but a great many of their lumina are partially clogged with mucilage. This occurs in large masses which take a dense haematoxylin stain. The length of the vessel segments varies considerably, fifty measurements giving a maximum of 760 mm., a minimum of 350 mm., with an average of 462 mm.

The walls of the vessels contain many bordered pits. These are arranged alternately, when the vessel wall is contiguous to water conducting elements, so that as a result the whole vessel is covered with diagonal rows of these pits. This alternation of pits is accompanied by crowding to such an extent that the pits, because of their mutual contact, become hexagonal. Bordered pits, in vessels that adjoin medullary ray cells, occur in isolated groups that are banded into vertical and horizontal rows. These groups usually contain nine pits in three rows. The pits on vessels that lie adjacent to water conducting elements are bordered on both sides of the middle lamella, but where the vessel is in contact with medullary ray cells, or wood parenchyma cells having simple pits, the pits in common with the two elements are half-bordered. The diameter of the pits in the vessel wall averages $10\ \mu$. The mouth of the pit is elliptical and is $6\ \mu$

wide. Occasionally, where the vessel joins with ray cells, there is a tendency for the pits to lose their borders. These pits sometimes become greatly elongated, and often anastomose, giving the vessel wall a scalariform appearance. SOLEREDER (18) found in the related genera *Echinocarpus*, *Elaeocarpus*, and *Sloanea* that the pits become simple and elongate in a similar manner. The cross walls of the vessel segments vary from transverse to strongly oblique. The terminal apertures of the vessels are always of the porous type.

WOOD FIBERS.—In transverse section the lignified wood fibers appear in wavy bands more or less concentric with the rings of growth (fig. 1). Several of these bands may occur within the limits of a single growth ring. Within these zones the fibers are arranged in uniform radial rows. Alternating with these lignified zones are similar bands of unlignified fibers, giving the whole a decidedly laminated appearance. The lignified areas make up approximately 60 per cent of the wood, while the remaining 40 per cent is composed of the parenchymatous areas. The wood fibers vary in length from 0.6 to 1.4 mm., their average length being 0.97 mm. The width of the medium portion is $32\ \mu$ and the thickness of the wall $7\ \mu$. The width of the lumina varies from 12 to $38\ \mu$, with an average of $25\ \mu$.

In a longitudinal section the typical characters of the fibers show to advantage. They appear as slender, elongate elements, spindle-shaped and sharp pointed. They run parallel to one another and exhibit very little, if any, interweaving. They are arranged in a distinct storied fashion.

The study of macerated material often shows the wood fibers with sawlike edges, due to the fact that these fibers are developed adjacent to the medullary rays, the cells of which have left their imprint on the fibers (fig. 18). A few fibers were found with forked ends; others were decidedly flattened.

In the walls of the fibers the pits are slitlike and extremely elongated (figs. 18, 26). They extend beyond the oval outline of the pit membrane, which is exceedingly narrow. In some fibers the outlines of the pit membranes do not coincide, but appear at right angles to one another, giving rise to a cross-sword appearance. The pits usually appear to be scattered, without any apparent regularity. Sometimes, however, they appear in isolated groups or even in longi-

tudinal rows. RECORD and others have called attention to the fact that these localized pit areas, upon the fibers, in some woods tend to make the ripple marks more distinct. The pits usually appear at an oblique angle to the long axis of the fiber (fig. 18).

MEDULLARY RAY.—The medullary rays are very pronounced and of two types, uniseriate and multiseriate, the latter being outnumbered by the former approximately two to one (fig. 2). The multiseriate rays are often six or seven rows of cells wide tangentially. In a transverse section the rays are seen to run continuously through the lignified and unlignified bands, and appear to widen tangentially within the lignified zone (fig. 1). In some areas near the pith, the rays appear to merge into the parenchymatous bands of tissue to such an extent that they are very difficult to delineate.

In tangential section the rays are from a few to many cells high (fig. 2). The multiseriate cells are often as high as seventy cells. That the rays are heterogeneous is clearly brought out by a study of the radial section. In this aspect the rays present a muriform appearance, consisting of many rows of cells. Some of these cells have their long axes extending in a radial direction, while others have their long axes extending vertically. In tangential view, in which the cross-section of the medullary ray is shown, these differentiated marginal cells are seen to occur as an outside layer of cells, practically investing the more numerous and smaller cells of the medullary ray as a sort of sheath. The rays vary in height from 0.15 to 2.65 mm., and in width from 0.02 to 0.09 mm. at the widest point. The individual cells of the ray, as measured in transverse section, vary in thickness from 7 to 21.3 μ , and from 48 to 105 μ in length, with an average of 65 μ . In height they vary from 35 to 166 μ , with an average of 50 μ .

All the cells of the medullary ray contain numerous simple pits where they join similar parenchymatous cells. Where they join conducting elements such as fibers and tracheids, the pits are half-bordered, being simple on the wall of the ray cell, but bordered on the wall of the conducting elements. The cells of the ray contain numerous large starch grains 8–10 μ in diameter. These have prominent hila. Mucilage is frequently present in the cells of the ray in small irregular masses.

WOOD PARENCHYMA.—In transverse section the wood parenchyma elements appear diffused throughout the growth rings, but are

most numerous around the vessels. In a longitudinal aspect, the wood parenchyma strands appear much like septate wood fibers, differing from these by their thinner walls and numerous, simple pits (fig. 32). The strands usually consist of four cells arranged end to end in vertical rows. The septations are transverse and the individual cells are prismatic, except the end ones, which are pointed. They measure $16-55\ \mu$ in width and $40-196\ \mu$ in height.

The number of pits in these elements varies. Where they are adjacent to medullary ray cells or vessels, the pits are more numerous than when they lie adjacent to fibers or to other wood parenchyma cells. The wood parenchyma elements are usually in seriation with the vessel segments.

Crystals of the rhombohedral type are common (fig. 29). These occur in chambered parenchyma strands, four to six to the strand. They measure about $20 \times 25\ \mu$. They are not found in material that has been treated with hydrofluoric acid.

FIBER TRACHEIDS.—Fiber tracheids are occasionally found in this wood (fig. 31). When present they usually are found adjoining the vessels. In transverse section they resemble small vessels. They are much thicker and shorter than the wood fibers, measuring about $55 \times 475\ \mu$. The walls are thinner than the wall of either the wood fibers or vessels, and are supplied with prominent bordered pits, not unlike those found in the walls of the vessel. These elements taper to a gently rounded point at the ends.

GROWTH RINGS.—The growth rings are irregular in occurrence, and do not correspond to annual periods of growth. They are in all probability due to a seasonal variation in climate causing a periodic growth in thickness of the stem. The rings exhibit considerable variation in width, and are demarcated by a band consisting of four to six rows of parenchymatous cells. These are continuous through the lignified and unlignified parenchymatous zones, and are to be distinguished from the other parenchymatous elements by a gradual reduction in their radial diameter.

PITH.—The pith is nearly round in transverse section, with a minutely crenulate outline. It averages 7 mm. in diameter, and is made up of isodiametric, polygonal parenchymatous cells whose diameter is about $150\ \mu$. The walls of the cells are thin, from 1.8 to $2\ \mu$ in thickness. The pith crown consists of five to eight rows of cells

that are somewhat smaller than the cells toward the center of the pith area. The cell walls have a slight secondary thickening, and are abundantly pitted with simple pits. No lignified cells are present. The pith is distinctly septate when seasoned; the openings between the diaphragms are narrow and extend beyond the inner edge of the pith crown. Mucilage cavities are numerous, often forty or more appearing in one transverse section. Those of the central region are round in transverse section and are arranged in an irregular fashion, while those of the pith crown are elliptical in transverse section and are arranged around the pith in the form of a circle. These cavities measure about $240\ \mu$ in diameter, and in most cases are clogged with mucilage. When a portion of the pith was removed and placed in water, strands of mucilage exuded until the water became thick like syrup.

Heliocarpus appendiculatus

LOCALITY AND HABIT OF GROWTH

The structure of the wood of this tree is so similar to that of *Heliocarpus popayanensis* that a detailed description is unnecessary, and emphasis will be placed chiefly upon differences. The tree is native to Central America and Mexico. It has been collected in Guatemala by JOHN DONNELL SMITH, in the State of Tabasco, Mexico, by LINDEN, and in Talemania, Costa Rica, by AD. TONDUZ. The wood specimen upon which this study is based was collected near San Jose, Costa Rica, in the summer of 1918. The plant differs from *H. popayanensis* in having leaves that are appendaged at the base. The trees have long, straight trunks, 25-30 cm. in diameter. The leaves are not unlike those of basswood in outline. The trees grow gregariously in the low hot lands as well as in the cooler uplands. The tree is called Burio Blanco in Costa Rica, and Macate in Guatemala. The former name means white wood, and the latter refers to the fibrous nature of the bark. These vernacular names are also applied locally to various other plants of the Tiliaceae.

GROSS ANATOMY

The wood of this tree, like *H. popayanensis*, is a light weight, diffuse porous, laminated wood, without pronounced taste or odor. The wood near the periphery weighs 15.8 pounds to the cubic foot,

when oven-dried at a temperature of 100° C. It has a specific gravity of 0.25. The wood near the pith weighs 9.67 pounds per cubic foot, and is much softer and less fibrous in nature than the later formed wood. Ripple marks are readily seen on the surface of the longitudinal cuts, and appear more prominent on the tangential than upon the radial surface. These marks occur on the average of twenty-six to the centimeter. They are caused by the tierlike rankling of the wood parenchyma strands. The wood fibers or cells of the wood parenchyma strands are not in seriation.

MINUTE ANATOMY

VESSELS.—The microscopic structure of this wood is similar to that of *H. popayanensis*. It has a laminated appearance when viewed in transverse section. It exhibits a similar difference in the type of wood found in the central part of the axis and that toward the periphery. The merging of the medullary rays into the bands of parenchymatous tissue is more noticeable than in *H. popayanensis*. No differences are observed in the size, shape, or markings of the vessels, except that an occasional scalariform perforation is found. In one case a vessel segment was found with such a perforation at one end and a porous perforation at the other (fig. 27). The vessels are fewer than in *H. popayanensis*. The wood fibers differ only in their greater length and slightly greater diameter, as shown by the following average measurements:

	Maximum	Minimum	Average
Length	60 μ	18 μ	37 μ
Width	2.43 mm.	0.756 mm.	1.37 mm.

No important differences are exhibited in the wood parenchyma elements of the two species. The medullary rays fall more clearly into the two types, uniseriate and multiseriate, there being few transitions between the numerous uniseriate and the high multiseriate rays. Mucilage is rarely found, and the presence of crystals has not been observed.

The species exhibits no semblance of annual rings of growth, differing sharply in this respect from *H. popayanensis*. Similar observations have been made in *Ochroma*. The Ecuador species of this genus show pronounced growth rings, while in the Central Ameri-

can species they are lacking. The cause of this phenomenon is probably physiological, and no doubt to be associated closely with the character of the climate of the two regions.

COMPARISON OF OUTER AND INNER WOOD IN *HELIOCARPUS*

The wood of most trees of temperate regions can be divided into two portions, heart wood and sap wood. It is well known that the former is usually harder, and in general of a darker color than the latter, due to the infiltration of gums, resins, and other substances into the walls of the cells of that region. It is equally well known that sap wood, in such cases, contains the only living elements of the wood, and that, generally speaking, it is less valuable commercially than the heart wood.

The study of two species of *Heliocarpus* has resulted in some divergences from these generalities. Technically, only discolored parts are called heart wood, and in this respect, in the two species of *Heliocarpus* studied, heart wood is lacking. There is, however, considerable difference in the hardness, density, and weight in the wood of the two regions, as is evident from the data given. They show that the wood nearest the periphery is in all cases harder and heavier than that nearer the pith, and that this change is not abrupt. It should be stated, however, that no increase in hardness or weight occurs until the end of the third season's growth.

The problem of accounting for the physiological change undergone, whereby harder, heavier, and denser wood is produced, is difficult of solution without direct experimental evidence. It has recently been pointed out by KRAUS and KRAYBILL (7), however, that a relative decrease of nitrates in proportion to the carbohydrates makes for fruitfulness, fertility, and lessened vegetation. These plants produce flower and fruit about the fourth year, and subsequently their growth is greatly retarded. Histological examinations show that the transition from the softer and lighter to the heavier and harder wood begins to take place about the time the tree flowers and fruits. This suggests that the greater density and degree of lignification of the wood might be due to an upset in the carbohydrate nitrogen relations undergone in the plant at the time of fruiting.

Although finding it difficult to determine any definite proportion

of the lignified and unlignified areas, in general, it can be said that the former become larger and compose more of the bulk of the tissue toward the periphery, while the latter correspondingly increase nearer the pith. These parenchymatous bands are sometimes 2 mm. thick tangentially in this region. They are composed of pithlike, short cylindrical cells, with their long axes extending vertically; while, as has just been shown, the similar areas in the wood near the periphery are composed of unlignified wood fibers. These conditions in the fundamental composition of the two regions account for the more fibrous nature of the wood toward the periphery, and for the absence of "ripple marks" in the wood nearer the pith. Infiltration of gums, mucilage, or other substances into the cell walls has not occurred, but in the region near the pith the lumina of the vessels often contain these substances. True wood parenchyma elements are far more numerous in the region near the periphery, and become fewer and fewer until they disappear entirely near the pith. Numerous measurements were made to ascertain whether the walls of the elements of the two regions showed any differences in thickness, but no noticeable differences were found to occur.

Apeiiba aspera

LOCALITY AND HABIT OF GROWTH

The wood sample of *Apeiiba aspera* was taken by Dr. H. E. STORK in Costa Rica during the summer of 1920. A specimen of the plant bearing fruit and leaves was also taken. The plant belongs to the tribe Apeibeae of the Tiliaceae, and is therefore closely related to *Heliocarpus*. The genus *Apeiiba* consists of six arboreal and shrubby species endemic in the hot countries of America. The members of this genus are characterized by the possession of discoid spiny fruit, 6-8 cm. in diameter. The species is widely scattered, having been collected not only in Costa Rica, but upon the Atlantic and Pacific sides of South America as well. It is found more abundantly in Costa Rica in the llanos and hills of the Atlantic side (9). It is a large symmetrical tree with a long cylindrical crown, gently rounded at the top. It attains a height of 40 m. and a diameter of 0.7 m. The natives call it Peine de Mico in Costa Rica. It is also known as Burio, but, as has been pointed out, this term is applied indiscriminately to

several species of the Tiliaceae, as well as to some few species belonging to other families.

GROSS ANATOMY

The wood of this tree is a diffuse porous, laminated, light weight wood (fig. 3). It is somewhat finer in texture than the wood of *Heliocarpus*, and similar to the latter in having no sharp line of demarcation between the wood of the central part of the axis and that toward the circumference. The wood near the pith is much softer, lighter, and more extremely laminated than that toward the periphery. There is a greater difference between the two regions than noted in *Heliocarpus*. The wood near the periphery, when oven-dried at a temperature of 100° C., weighs 19.5 pounds to the cubic foot, and has a specific gravity of 0.31; while that near the pith runs as low as 10.13 pounds to the cubic foot and has a specific gravity of 0.16. It is evident from this that the weight of any given block of wood will vary considerably, depending upon the proportion of wood from the two regions which it contains.

The wood is light brown, odorless, and tasteless. "Ripple marks" are numerous on both the radial and tangential surfaces, and can be seen easily with the unaided eye. These ripple marks are more numerous on the tangential than upon the radial surfaces. As noted in *Heliocarpus*, these ripple marks are due to the tierlike ranking of the wood parenchyma strands, and appear to be a constant feature attending all investigated woods of the Tiliaceae. Growth rings are visible to the unaided eye, and are extremely variable in width. The medullary rays do not continue across the parenchymatous zones in the region near the pith, but lose their identity completely as they merge into the parenchymatous tissue of this region. They reappear, however, when the next zone of lignified tissue is reached, and run through this uninterrupted.

MINUTE ANATOMY

VESSELS.—In a transverse section, as in fig. 3, the vessels are seen to be evenly distributed throughout the wood. These are usually scattered singly, or in radial rows consisting of three or four vessels. The average radial diameter is 250 μ , while the average tangential diameter is 175 μ . The length of vessel segments, as obtained from

measurements of numerous isolated elements, is maximum 0.843 mm., minimum 0.320 mm., with an average of 0.510 mm.

The pores are oval in outline, or in cases where two or more vessels join, the contiguous tangential walls are flattened in a radial direction (fig. 3). The walls of the vessels are 8–12 μ thick, strongly lignified, and are marked by numerous bordered pits (fig. 22). These pits average 32 μ in diameter, and their arrangement and shape are not different from those noted in the vessels of *Heliocarpus*. The cross walls of the vessels are mostly transverse, but an occasional oblique one is found. The terminal apertures are porous and the annular ridges are extremely narrow (fig. 4). Mucilage is present in small globules in some of the vessels.

WOOD FIBERS.—The wood fibers are usually longer than those of *Heliocarpus*. They vary in length from 0.6 to 1.9 mm., and average 1.23 mm. Their average width, at the widest point, is 35 μ . The ends of the fibers are often forked, and sometimes the margin of the fiber toward the end presents a sawlike appearance, due to the pressure exerted by the adjoining medullary ray cells. The longitudinal course of the fibers is greatly distorted, due to the pressure of very "fat" medullary rays (fig. 4). Within the lignified areas the fibers are lignified in various degrees, grading from unlignified fibers to strongly lignified fibers. The thickness of the cell walls of the fibers varies between 5 and 8 μ , the average being about 6.5 μ . Numerous bordered pits are present. These are slitlike and are elongated horizontally (figs. 17, 19). Usually their long axis lies obliquely to that of the fiber. The border is very much reduced and the pits frequently simulate simple pits. A great many septate wood fibers are present. Ordinarily there is not more than one septum to the fiber, but occasionally two or more are present. These measure 2 μ in thickness and stain densely with haematoxylin.

MEDULLARY RAYS.—The enormous medullary rays in this wood are perhaps its most striking character, and are shown to best advantage in a tangential section (fig. 4). In this aspect they are seen to be, in most cases, seven or eight cells wide tangentially. Occasionally a uniseriate ray is found, but these are not numerous. The average height of the rays is 0.83 mm., but rays as high as 2.0 mm. are occasionally found. The cross-section of the ray cells (tangential

section of wood) shows them to be practically alike in size and shape. They are $30\ \mu$ in width and are practically circular in outline. The cell walls are about $7\ \mu$ thick, and contain numerous circular, simple pits. A radial section of the wood shows to advantage the heterogeneous structure of the ray. The marginal cells have their greatest diameter vertically. In some rays the majority of the cells composing the ray appear to be similar, while in other rays there is a gradual transition between the type of cells of the marginal row, to procumbent cells within the body of the ray. No difference exists as regards the walls, pitting, or contents of the ray cells of the margin and those within the ray, and therefore these cannot be regarded as true marginal ray cells such as are found in *Nyssa*, *Aesculus*, and *Salix*. As ascertained from radial sections, the upright ray cells average 0.066 mm. radially and 0.058 mm. vertically. The procumbent ray cells average 0.087 mm. radially and 0.041 mm. vertically. The ray cells contain numerous hexagonal crystals and tannin granules. The crystals average $24\ \mu$ in height and are $17.5\ \mu$ wide. The cells contain large nuclei and are rich in other protoplasmic contents.

WOOD PARENCHYMA.—The wood parenchyma is diffuse and is found abundantly throughout the stem. Its strands are 0.3–0.5 mm. in length, and consist of four perpendicular cells, although occasionally a strand consists of six or seven cells (fig. 16). The strands are not in seriation with the vessel segment or wood fibers, neither are the individual cells of adjoining fibers in seriation. The average width of a wood parenchyma cell, tangential diameter, is $22.5\ \mu$. The wall is $3.5\ \mu$ thick and abundantly pitted with simple pits. The height of the individual cells of the strand averages $76\ \mu$. The cells retain their protoplasmic contents indefinitely. No crystals are present. The walls of the wood parenchyma cells are sometimes slightly lignified, and are easily confused with the septate wood fibers.

GROWTH RINGS.—The rings of growth in this species are much more distinct than in *Heliocarpus*, but evidently do not represent true annual increments of growth. They are delimited by a single row of wood parenchyma strands, and are very variable in their circumference. They are considerably wider in the central part of the axis, and become gradually narrower toward the periphery.

BARK.—The bark of a small trunk 10 cm. in diameter measures 7 mm. in thickness. It is very tough and fibrous, and is much harder and heavier than the wood. The color is cinnamon brown, and it is covered with small wartlike projections. A transverse section shows it to be filled with large irregular cavities, containing mucilage in profuse quantities. These cavities are sometimes 3 mm. or more in diameter.

Pachira barrigon

LOCALITY AND HABIT OF GROWTH

Pachira (2) is a genus of the Bombacaceae, a family noted for the great quantity of light weight wood which it produces. To this family belongs *Ochroma*, which has nine species, all of which produce the light weight wood known commercially as Balsa. *Pachira* includes about forty species, all endemic to tropical America. *P. barrigon* is the largest species of the genus, the trees often reaching a meter or more in diameter. They grow gregariously in Panama and Costa Rica, and are known as the "big bellied" trees, due to the peculiar enlargement of the trunks a few feet above the ground. The plants reproduce freely from cuttings, and are used locally in producing fences and hedges. The wood is used extensively for forms in concrete construction work.

GROSS ANATOMY

This wood is one of the most important of the tropical light weight woods, and resembles "balsa" more closely than the other woods discussed. It is homogeneous, and is composed approximately of 90 per cent parenchyma. It is diffuse porous. The pores are relatively larger and fewer in number than in the other woods studied. The wood is tasteless and odorless, and weighs 10 pounds to the cubic foot when oven-dried. It has a specific gravity of 0.16. "Ripple marks" are visible on the tangential and radial surfaces. These are due to the arrangement of the wood parenchyma strands in radial and tangential seriation, and to a similar arrangement of the cells that compose the strands. These marks are very much smaller and more numerous than those found in the other woods discussed in this paper. There are, on the average, seventy of these ripple marks to the centimeter.

MINUTE ANATOMY

VESSELS.—The vessels as exhibited in a transverse section are oval in outline, with their long axis extending in a radial direction (fig. 5). The average width of the vessels in tangential diameter is 249 μ , and their average width in the radial diameter is 355 μ . The walls vary in thickness in different vessels, but average 7.2 μ . Tyloses and mucilage are lacking in the vessels. The variation exhibited in the length of the vessel segments is maximum 0.498 mm., minimum 0.192 mm., with an average of 0.328 mm.

The pitting in the walls is like that described for *Heliocarpus popayanensis*, except that the closing membrane becomes greatly elongated horizontally, and is very narrow vertically. The pits are so crowded that a hexagonal outline to the border is the rule. The diameters of these pit borders average 10 μ , while the size of the pit mouth is 3.5 \times 6.5 μ . The cross walls of the vessel segments are generally transverse, but occasionally one is found that is slightly oblique. The terminal apertures are always porous, and have pronounced annular ridges.

WOOD FIBERS.—The fibers are arranged irregularly within the parenchymatous tissue (fig. 5). They are variable, often extremely long, ranging in length between 0.664 and 2.59 mm., with an average of 1.23 mm. The average width of the fibers at the point of their greatest diameter is 35 μ , while the thickness of the wall is 7 μ (figs. 24, 25). The pits are reduced bordered pits with elliptical mouths. In some fibers they are greatly reduced in number. The border averages 7 μ in diameter. In macerated material some of the fibers are seen to possess serrated edges and forked ends. The latter are usually needle-like, but are often flattened and bent. The margins of the fibers in many cases are permanently indented, due to the turgidity of the adjoining parenchymatous cells (figs. 24, 25). The indentations are always opposite, because of the seriation, and correspond in their vertical extent to the height of these parenchymatous cells. Septate fibers are frequent in this wood. There is usually but one septum within these fibers, but occasionally there are two or more. These are 1.5 μ in thickness, and have a different chemical nature from the walls of the fiber, as exhibited by their greater affinity for haematoxylin stain.

WOOD PARENCHYMA.—Wood parenchyma makes up the bulk of the wood. As has been pointed out, the wood fibers are irregularly interspersed within this parenchymatous tissue. The wood parenchyma strands are about 0.5 mm. in length, and typically consist of four cells. These cells average $45\ \mu$ wide tangentially, and give rise, because of this, to many intercellular spaces. As is typical of wood parenchyma strands, the tip cells taper to a somewhat rounded point, which interlocks between the tip cells of the two adjoining strands. The numerous simple pits are almost circular in outline, and are about $7\ \mu$ in diameter. The cell walls are thin, averaging about $3.5\ \mu$. No crystals are present, but many of the cells contain mucilage, tannin granules, and starch grains.

MEDULLARY RAY.—The medullary rays are far less prominent than in *Apeiba*. Both uniseriate and multiseriate rays are found; the former are of very limited occurrence, while rays two or three cells wide is the rule (fig. 6). Rays more than four cells wide never occur. The cells of the medullary ray differ somewhat in size and shape, those toward the margin being somewhat shorter radially and wider perpendicularly than those away from the margin. The cell walls are $3.5\ \mu$ thick, and contain numerous simple pits $3\text{--}7\ \mu$ in diameter. In a radial section the cells average $35\ \mu$ in height, and vary from $40\text{--}125\ \mu$ in length. Their third dimension, as ascertained by averaging the measurements obtained from fifty cells in transverse section, is $22\ \mu$. The rays are not in seriation with the wood parenchyma strands, but a considerable number end at the same level, as is shown in tangential section (fig. 6). In this section the height of the individual rays is easily obtained. Some of these attain the height of 1.6 mm., but the average height was 0.830 mm. Large starch grains $8\text{--}10\ \mu$ in diameter occur profusely in the cells. Cells containing tannin granules are numerous, but crystals are lacking.

GROWTH RINGS.—The growth rings are unusually prominent for a light weight tropical wood, due to the presence of dark brown lines. These appear on the face of the summer wood, and clearly delimit the season's growth. The dark line is due to the greater number of lignified fibers in this region. The growth rings average 1.5 mm. in width near the pith, but toward the periphery they become narrower, and are usually less than 0.5 cm. wide.

Cavanillesia platanifolia

LOCALITY AND HABIT OF GROWTH

Cavanillesia is a genus of the Bombacaceae, being closely related to *Pachira* and *Ochroma* (17). The genus consists of two species in South America, one in the eastern and one in the western part. *C. platanifolia* extends northward into Panama, eastward into Colombia, and southward into Peru (10). The trees have huge, straight trunks, 2-3 m. in diameter, and very small, flat crowns. The few branches are usually given off near the top of the tree. They are called Quipo in Panama, and Maconda in northwestern Colombia. These trees have very light weight wood, which has been proposed for commercial use as a substitute for cork, and as an insulating medium under the name of "quipo" or "cuipo." The bark of the tree appears to be the chief source for mechanical support. This is two or three inches thick and is tough and fibrous. Workmen are very wary about cutting these trees, on account of their immense weight and the difficulty of telling which way they will fall. It is said that if the bark be cut entirely around a tree it will fall of its own weight. There are no data available as to rate of growth, but the histological appearance of the wood indicates that it grows rapidly. The trees are deciduous, the leaves being off in the dry season, December to May. The wood specimen upon which this study is based was collected near Pedro Muguel, Panama, in 1918.

GROSS ANATOMY

The wood of this tree is diffuse porous, soft, light, and weak. It is light yellow, odorless, and tasteless. When green the wood is much heavier than water, but when kiln-dry it is the lightest wood known, with a weight of 6.2 pounds per cubic foot and a specific gravity of 0.10. The wood from the periphery may weigh as much as 11 pounds to the cubic foot. The vessels are relatively large and visible to the naked eye. They are rather regularly distributed, but become more numerous toward the outer limit of the growth rings. The medullary rays are visible without a lens, and appear somewhat lighter in color than the rest of the wood. They are very large and prominent on the radial surface. Growth rings are prominent and are limited by a

light colored line. Very fine indistinct "ripple marks" are present on the longitudinal surfaces. These are caused by the horizontal seriation of the parenchyma cells. The medullary rays are not storied, but there is a tendency for the vessel segments to appear in seriation.

MINUTE ANATOMY

VESSELS.—In transverse section the vessels occur singly or grouped into clusters containing two to seven vessels. When single the vessel is oval in outline, averaging $200\ \mu$ in diameter tangentially, and $350\ \mu$ in diameter radially (fig. 11). The average thickness of the wall is $6\ \mu$. The wall is heavily lignified and richly supplied with bordered pits measuring $11\ \mu$ in diameter. The pit mouth measures $3 \times 10\ \mu$, and has its long axis horizontally. These pits are arranged alternately, and usually have a circular border, which, however, often becomes hexagonal by mutual compression. Where the vessel adjoins the cells of the medullary ray the pits are half-bordered. The vessel segments are simple porous with nearly transverse cross walls. The annular ridge is not prominent, usually being less than $6\ \mu$ wide. Measurements taken of the length of fifty vessel segments gave a maximum of $0.750\ \text{mm.}$, a minimum of $0.640\ \text{mm.}$, with an average of $0.675\ \text{mm.}$

WOOD FIBERS.—The wood fibers are very few, and are generally isolated in and completely surrounded by parenchymatous tissue (fig. 11). They are more lignified, much smaller in diameter, and have much thicker walls than the parenchyma cells by which they are surrounded. In a transverse section it is very unusual to find two or more of these fibers with contiguous walls. The condition of the fibers in their relation to the surrounding parenchyma is similar to that noted in the wood of *Pachira*, except that the fibers are many times more numerous in the latter wood, as is evident by a comparison of figs. 5 and 11. The outline of the fibers in transverse section is hexagonal. Their average diameter is $25\ \mu$, and their average length is $0.85\ \text{mm.}$

The distribution and relative bulk of the fibers in relation to the parenchyma tissue are interesting, and in this regard numerous counts were made. A transverse section was divided into areas $10\ \text{mm.}$ square, and counts of the fibers appearing in each square gave

minimum 0, maximum 28, and average 16. From these data it is apparent that the number of fibers is extremely variable in different areas of the stem, and that they never make up more than a very small proportion of the bulk of the wood.

Reduced bordered pits are present in the walls of the fibers. These are slitlike, $1.5 \times 6 \mu$ in diameter, and their long axis is oblique to that of the fiber (fig. 21). Upon the inner walls of the fibers definite spiral or scalariform thickenings are present. These do not extend the length of the fibers, but occur here and there along its axis. The fibers are usually septate, and never appear in the parenchymatous sheath immediately surrounding the medullary rays.

WOOD PARENCHYMA.—The wood parenchyma is paratracheal, and is composed typically of four cells arranged perpendicularly into a strand. These cells are usually flattened radially. Their average is $15 \times 45 \mu$, and their height 60μ . The cell wall measures 2μ , and the numerous simple pits marking the wall average 3.5μ in diameter. Plain parenchyma, similar to the wood parenchyma, but with much larger cells, makes up the bulk of the wood. In a transverse section these cells are usually hexagonal and have a greater radial than tangential diameter, measuring $63 \times 105 \mu$ respectively. The walls measure 1.5μ in thickness, and contain an occasional small simple pit 3.5μ in diameter. In a longitudinal section these cells resemble pith cells. They average 128μ in height (fig. 12). Microchemical tests for lignin showed, contrary to expectation, that the walls of these cells are slightly lignified throughout. The light weight of the wood is to be correlated with the large lumina of these parenchyma cells, their thin walls, and their relatively great numbers, together with the relatively small number of wood fibers.

MEDULLARY RAY.—The medullary rays are usually multiseriate, but an occasional uniseriate ray occurs. In transverse section they are rather regularly distributed, but are variable in width throughout their course, appearing multiseriate, then uniseriate, or perhaps disappearing entirely, only to reappear some distance beyond. On either side of the medullary ray is a parenchymatous zone two or more cells wide, in which no wood fibers are to be found. In tangential section the estate of these cells is more easily seen. They surround the smaller cells of the ray as a sheath, the outside margin of

which is distinctly spindle-shaped, and which is usually bounded by the slender wood fibers which weave between the spindle-shaped masses (fig. 12). The parenchyma cells of the sheath often pass into the ray, making its margin very irregular and often dividing it into two or more areas. In a radial view the ray is seen to be heterogeneous, and appears very extensive, making up approximately 50 per cent of any given area.

The ray cells are much smaller than the parenchyma cells of the sheath, and have somewhat thicker walls. As ascertained from a tangential section, the cells average $35\ \mu$ in vertical diameter and $22\ \mu$ in tangential diameter. The simple pits in the walls of the medullary ray cells average $5\ \mu$ in diameter. The ray cells contain starch, while the cells of the sheath often contain mucilage in profuse quantities.

GROWTH RINGS.—The growth rings are prominent, and are limited by several rows of parenchymatous cells that are narrower radially and generally smaller than the cells of the other portions of the ring (fig. 11).

Wercklea insignis

LOCALITY AND HABIT OF GROWTH

Wercklea is a genus of the Malvaceae, recently established by PITTIER and STANDLEY (19). It is a member of the subfamily Hibiscaceae, and is most closely related to *Hibiscus*. *W. insignis* is the only species of the genus, and is confined to the northwest slope of the Central Cordilleras in Costa Rica, the type locality being the pass known as La Palma, between the volcanoes of Irazu and Barba. The largest specimens are said to grow on the lower grounds north of and below the pass. It has large showy flowers resembling those of the hollyhocks. A few specimens have been planted as ornaments on the grounds of Dr. JIMENEZ in Santa Jose, Costa Rica. The flowers are ephemeral, usually fading the day they blossom. For this reason the natives call the tree "Flor de dia." The tree reaches a height of 10 m., and has a trunk 30-40 cm. in diameter. Our specimen was collected in the type locality in 1918, and according to the collector the tree appears to be a rapid grower, but is not abundant. The collector tried repeatedly to germinate the seeds of this plant but was unsuccessful.

GROSS ANATOMY

The wood of this tree is a homogeneous light weight wood with unusually fine texture. It is ivory colored, odorless, and tasteless. When oven-dry it weighs 15 pounds to the cubic foot and has a specific gravity of 0.24. The pores are irregularly distributed throughout, but appear to be more numerous in some sections of the stem than in others. No growth rings are present, and there is no differentiation in color between the heart wood and sap wood. The latter, however, is somewhat lighter than the former. The medullary rays are visible to the unaided eye, are rather fine, and somewhat lighter in color than the rest of the wood. "Ripple marks" are visible on the radial surface. There are approximately fourteen of these to the centimeter, and the distance between the marks is fairly constant. The bark is thick, tough, and fibrous.

MINUTE ANATOMY

VESSELS.—The vessels are few in number and are distributed irregularly throughout the wood. In a transverse section of the wood the pores appear oval in outline, with their greatest diameter in a radial direction. Often four or five of these pores appear aggregated into a cluster with no regular arrangement (fig. 7). The walls average $4\ \mu$ in thickness, and are more strongly lignified than the walls of the wood fibers. The numerous bordered pits on the vessels are arranged alternately. The borders are generally circular, but often become hexagonal by mutual contact. The diameter of the pit border is 10–12 μ , while the mouth of the pit, which is elliptical, measures $7 \times 3.5\ \mu$. The fusion of these pits in some vessels gives rise to a scalariform appearance, and in other vessels the borders disappear and give rise to simple pits, extremely variable in size. The vessel segments are unusually short, varying in length between 0.3 mm. and 0.6 mm. in radial diameter. They average 0.24 mm., and in tangential diameter 0.144 mm.

WOOD FIBERS.—The fibers in this wood are its most striking character (figs. 20, 33). They differ in many respects from the fibers of the other woods investigated. The walls are very thin, averaging $3.5\ \mu$, weakly lignified, and contain numerous reduced bordered pits,

which are elliptical and about $4\ \mu$ in diameter. The fibers are extremely short and stubby. Their average width is $60\ \mu$ and their average length is $0.8\ \text{mm}$. It is not unusual to find fibers that are $0.1\ \text{mm}$. or more in width. They are more or less uniform in width throughout their greater length. In most cases they do not have long tapering ends, but come more sharply to a rounded point. The fibers are not definitely storied, but a tendency toward such a condition is evidenced in radial view. In transverse section the lumina are very irregular in size and shape. The outline of the fiber is usually hexagonal (fig. 7). Macerated material often shows fibers with serrate margins and forked ends.

MEDULLARY RAY.—The medullary rays are very similar to those described for *Heliocarpus*. Both uniseriate and multiseriate rays are present, the former occurring more frequently than the latter. The multiseriate rays are never more than 6-seriate. In a tangential section (fig. 8) they appear to be composed of cells of about the same diameter tangentially and vertically. These are circular in outline, and when the face wall appears in the section they seem to be abundantly supplied with simple pits $1.9\ \mu$ in diameter. The broad rays may be $2\ \text{mm}$. in height. They are not usually multiseriate throughout their entire height, however, but trail off into long uniseriate ends. Not infrequently these uniseriate portions connect the ray with similar multiseriate rays above or below. By a study of the radial section the ray is seen to be heterogeneous. The upright cells average $52 \times 166\ \mu$, while the average of the procumbent cells is $60 \times 112\ \mu$. The average thickness of the cell walls is $4\ \mu$. The cells do not contain starch, mucilage, or other contents.

WOOD PARENCHYMA.—The parenchyma is chiefly of the paratracheal type, but is also quite abundantly diffused throughout the wood. Each strand is composed typically of two perpendicular cells, which measure approximately $60\ \mu$ in diameter and $256\ \mu$ in height (fig. 30). The wood parenchyma strands are in seriation; this is especially noticeable in radial section, where the septations of the strands form a broken line across the section in a radial direction. This line serves to make the "ripple marks" stand out very prominently on the radial surface. The cell wall averages $4\ \mu$ in thickness,

and is richly supplied with simple pits which are approximately $2\ \mu$ in diameter. No crystals or other contents are found in the wood parenchyma cells.

Cordia heterophylla

LOCALITY AND HABIT OF GROWTH

Cordia is a genus of the Boraginaceae, subfamily Cordioideae. Its species are common and widely distributed in the tropical regions of both the eastern and western hemispheres, more than two hundred species having been described for the genus. *C. heterophylla* was described by RÖMER and SCHULTZ from material collected in French Guiana. The tree from which the wood specimen for this study was taken was found in the La Palma Pass, Costa Rica, between the volcanoes Barba and Irazu. It was a tree 30 cm. in diameter. It is not abundant and probably is not of great economic importance. The vernacular name applied to the plant by the natives is Zopilote.

GROSS ANATOMY

This is a light weight, laminated, straight grained, diffuse porous wood. The wood is odorless, tasteless, and light brown. Growth rings are visible, but they are very irregular in width. The medullary rays are fine and rather uniform in size and distribution. The pores often appear in prominent tangential rows. These are connected by tangential bands of wood parenchyma that are clearly visible to the unaided eye. There is no apparent differentiation between the wood of the central portion of the stem and that toward the periphery in color, weight, or density. The wood when oven-dry weighs 17 pounds to the cubic foot, and has a specific gravity of 0.27. No "ripple marks" are present on either the tangential or radial surfaces. The bark is light brown, and is extremely tough and fibrous. It is noticeably much heavier and harder than the wood.

MINUTE ANATOMY

VESSELS.—By a study of a transverse section, such as is shown in fig. 9, the pores of the vessels are seen to be irregularly distributed, but appear to be more numerous within the zone of the last formed wood of each growth ring. The vessels appear singly, or more commonly aggregated into groups consisting of 2-8 vessels that are vari-

able as to size and shape. In case the vessel appears singly, the transverse outline is nearly circular. The walls of the vessels average $5\ \mu$ in thickness, and are abundantly supplied with bordered pits. These are arranged alternately, and in most cases have elliptical borders. The borders average $4.5 \times 8\ \mu$ in diameter, and surround similar elliptical pits $2 \times 6.7\ \mu$ in diameter. The pits have their greatest diameter in a horizontal direction. The tendency of the bordered pits of the vessels to lose their borders, thereby giving rise to simple pits, has not been observed in this wood. The vessel segments are always simple porous, and are extremely short in proportion to their width. The nearly transverse cross walls have almost disappeared, leaving only extremely narrow annular ridges, often less than $7\ \mu$ wide. The results obtained from the measurements of fifty vessel segments were as follows:

	Maximum	Minimum	Average
Length.....	365 μ	100 μ	276 μ
Width.....	330 μ	100 μ	224 μ

WOOD FIBERS.—A transverse section shows the lignified wood fibers in isolated groups usually surrounded by parenchymatous tissue (fig. 9). These groups are more or less rectangular in outline, and have a fairly definite arrangement in bands, both in a tangential and a radial direction. This is due to the blocking out of these groups by the crossing, at right angles, of the broad medullary rays with the prominent tangential bands of wood parenchyma. A detailed examination of any one group of wood fibers, in transverse section, shows that the lumina of the fibers vary from extremely large to very minute ones. This is due to the fact that the fibers are not storied, so that a transverse section not only cuts the enlarged portion of some fibers, but the tapering smaller ends of many fibers also. These small openings shown among the lumina simulate intercellular spaces, but are readily distinguished from the latter by the fact that their walls are secondarily thickened. The fibers vary considerably in the degree of lignification of their walls, some appearing extremely lignified and others less so. The fibers vary from circular to hexagonal in outline. In general they are circular near the enlarged middle portion, but become hexagonal along the tapering ends (figs. 23, 24). The bordered pits are not numerous. They often appear in isolated

groups, usually near the widest part of the fiber. They measure 2.4μ in diameter, their long axes being slightly oblique to the long axis of the fiber. The average results obtained from the measurements of fifty wood fibers were as follows: width of fiber at point of greatest diameter 43.3μ ; length of fiber 913μ ; thickness of cell wall 5μ .

MEDULLARY RAY.—The medullary rays are in most cases 3, 4, or 5-seriate. Uniseriate rays are occasionally found. In tangential section the rays are distinctly spindle-shaped, with bluntly tapering ends. The individual cells of the ray in this section are hexagonal in outline (fig. 10). The cell walls average 5μ in thickness, and contain numerous simple pits 1.5μ in diameter. The rays are often 2 mm. or more in height, but average 1.12 mm. The average diameter of the cells of the medullary ray in tangential section is 25μ . In transverse section the rays appear to be similar to those observed in the other light weight woods, and are heterogeneous in nature. The vertically elongated cells average $55 \times 58 \mu$ and the procumbent cells average $34 \times 115 \mu$. In transverse section the cells of the medullary ray are often wider tangentially than radially. This unusual condition has been reported previously by SOLÉREDER (18) in the woods of *Durio*, *Boschira*, and *Neesia*. Many small globules of mucilage occur in the cells of the medullary rays.

WOOD PARENCHYMA.—Wood parenchyma occurs abundantly, forming broad zones around, and tangential bands between, the vessels. These bands, composed largely of wood parenchyma alternating with the zones of lignified wood fibers, give the wood its characteristic laminated appearance. The wood parenchyma strands are usually hexagonal in transverse section, but are often flattened in a radial direction. They average 25μ in diameter. In longitudinal section the wood parenchyma strands appear to be made up of two perpendicularly placed cells with gently tapering ends (fig. 28). These cells average 0.2 mm. in length. Their walls are 3.5μ thick and contain numerous circular simple pits 2μ in diameter. The cells contain many small globules of mucilage. The strands of individual cells of the strands are never in seriation.

General observations

Of the woods studied, *Pachira barrigon* more closely resembles balsa. Its fibers are lignified to a greater degree than *Heliocarpus*,

Apeiba, *Ochroma*, or any of the other tropical light weight woods which the writer has examined. This, together with the fact that the fibers are extremely long, and are often interlocked, would account for the seemingly greater strength and rigidity of form than exhibited by many of these other woods. This would tend to make it a more desirable wood than *Ochroma* for commercial purposes, where strength plus lightness is desired. It consists of approximately 90 per cent parenchymatous tissue, with extremely thin walls and large lumina, factors that have made balsa famous for its light weight, and at the same time have made it excellent material for insulating and buoyant products.

The anatomical composition of this wood indicates that it is equal to balsa in these respects, and, in addition, that weight for weight it is a much stronger wood. The importance of this can be realized better when it is known that for many purposes it is necessary to veneer balsa with a heavier wood to increase its strength, and to treat it with various chemicals to ward off attacks of microorganisms. The writer believes that in the wood of *Pachira* the desired weight can be obtained, together with a stronger and more enduring wood. These factors, when considered with the facts that this wood is available in large quantities, that the tree is a rapid grower, and that the species reproduces freely from cuttings, indicate that this tree should become of great commercial importance as the demand for light weight timber increases.

Summary and conclusions

1. The commercial importance of light weight woods was demonstrated during the recent war. The economic use of these woods is increasing, as their value for insulating refrigerator cars, cold storage plants, and iceboxes becomes recognized.
2. Trees producing light weight wood appear to be confined to certain geographical areas. These areas in general are the continuously hot and humid regions of Central and South America. The trees appear in greater numbers and obtain their best growth in Costa Rica and Panama.
3. Taxonomically the majority of trees producing light weight wood are closely related, in nearly all cases belonging to the Tiliaceae, Malvaceae, and Bombacaceae of the order Malvales.

4. The woods of seven species of light weight timber producing trees are treated histologically. These species are *Heliocarpus popayanensis*, *Heliocarpus appendiculatus*, and *Apeiba aspera* of the Tiliaceae; *Wercklea insignis* of the Malvaceae; *Pachira barrigon* and *Cavanillesia platanifolia* of the Bombacaceae; and *Cordia heterophylla* of the Boraginaceae.

5. Two distinct types of wood are found among the tropical light weight woods, namely, laminated and homogeneous. Of the woods discussed, the former type is represented by *H. popayanensis*, *H. appendiculatus*, *A. aspera*, and *C. heterophylla*; while the latter type is represented by *P. barrigon*, *W. insignis*, and *C. platanifolia*.

6. Histological evidence indicates that the homogeneous type of wood is better adapted for insulation products than the laminated type.

7. The wood of *Ochroma* has been the principal wood used for these products during the past few years. The histological examination of the wood of *Pachira barrigon* supports the conclusion that this would make an excellent substitute. The tree grows rapidly, attains an immense size, and reproduces freely from cuttings.

8. The woods of *Heliocarpus popayanensis* and *Apeiba aspera*, because of their greater specific gravity, fibrous nature, and laminated structure, are less valuable for buoyant materials than *Ochroma* or *Pachira*, and could not be obtained in such quantities.

9. *Cavanillesia platanifolia* produces wood known as "Quipo." This is the lightest wood known and will no doubt become of great economic importance as the demand for light weight wood increases. Trees 2 m. or more in diameter are available in almost unlimited quantities.

10. In the laminated light weight woods investigated, there is a decided tendency for the medullary rays to merge imperceptibly into the areas of parenchymatous tissue, but they regain their identity when the next lignified zone is reached and pass through this uninterrupted.

11. *Wercklea insignis* and *Cordia heterophylla* produce light weight wood of excellent quality, that have a specific gravity low enough to make them suitable for buoyant products. The trees, although not abundant, grow rapidly, and could be produced in a comparatively short time.

12. A distinct difference between the wood produced near the pith and that nearer the periphery is noted in all species investigated: the latter being harder, heavier, and generally more fibrous than the former.

13. The walls of the individual elements do not vary in thickness in different regions of the stem; but the thicker walled lignified wood fibers become relatively more abundant toward the circumference, while the number of thinner walled parenchymatous elements correspondingly increases toward the pith.

14. A correlation between the upset in the carbohydrate-nitrogen relation at the time of fruiting and the differences in density and weight of the wood produced in different areas of the stem is suggested.

15. Light weight woods are fundamentally very similar to heavier dicotyledonous woods. They differ from the latter principally in being composed of thinner walled elements with proportionally larger lumina, a characteristic which accounts for their light weight and low specific gravity.

16. The following characteristics appear to be common to all species producing light weight wood: (1) they are plants with very large leaves that are soft in texture; (2) the bark is thick, strong, and fibrous; (3) the wood is white or very light in color.

Grateful acknowledgment for assistance and suggestions is made to Professor W. W. ROWLEE, under whose immediate direction the work was performed, and to Professors D. REDDICK, H. D. REED, and S. N. SPRING, for help and criticism in the preparation of the manuscript.

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EXPLANATION OF PLATES XXVI-XXVIII

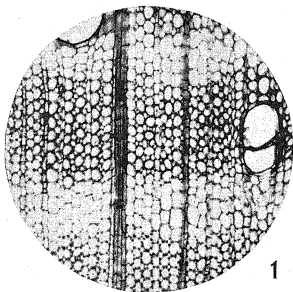
All photomicrographs show magnification of 50 diameters.

PLATE XXVI

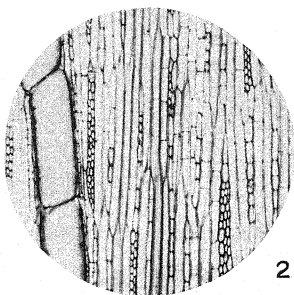
- FIG. 1.—Transverse section of wood of *Heliocarpus popayanensis*.
FIG. 2.—Tangential section of same.
FIG. 3.—Transverse section of wood of *Apeiba aspera*.
FIG. 4.—Tangential section of same.
FIG. 5.—Transverse section of wood of *Pachira barrigon*.
FIG. 6.—Tangential section of same.

PLATE XXVII

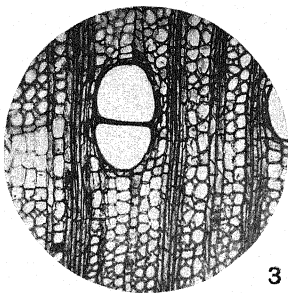
- FIG. 7.—Transverse section of wood of *Wercklea insignis*.
FIG. 8.—Tangential section of same.
FIG. 9.—Transverse section of wood of *Cordia heterophylla*.
FIG. 10.—Tangential section of same.
FIG. 11.—Transverse section of wood of *Cavanillesia platanifolia*.
FIG. 12.—Tangential section of same.



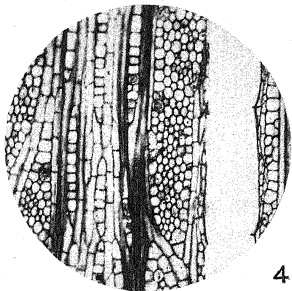
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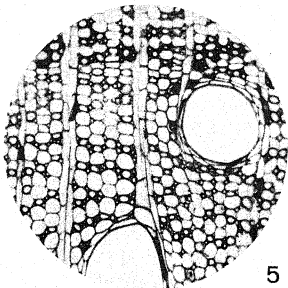
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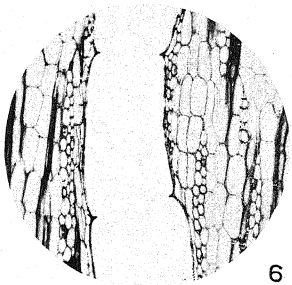
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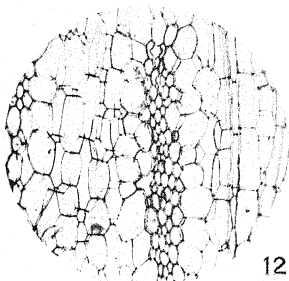
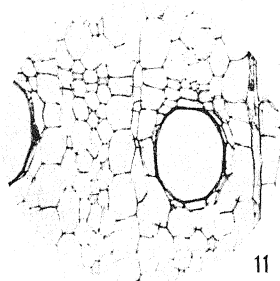
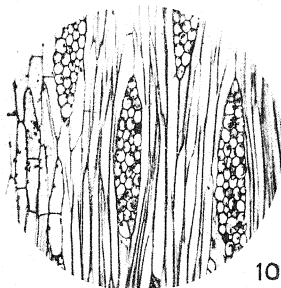
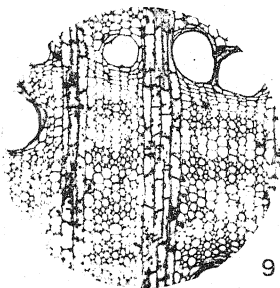
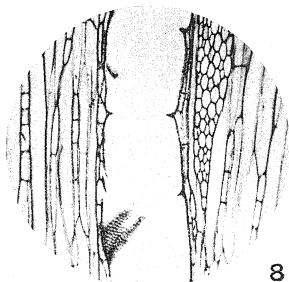
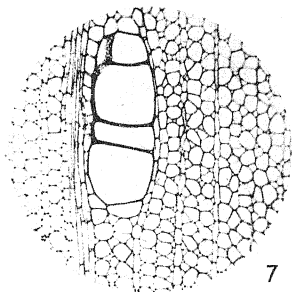


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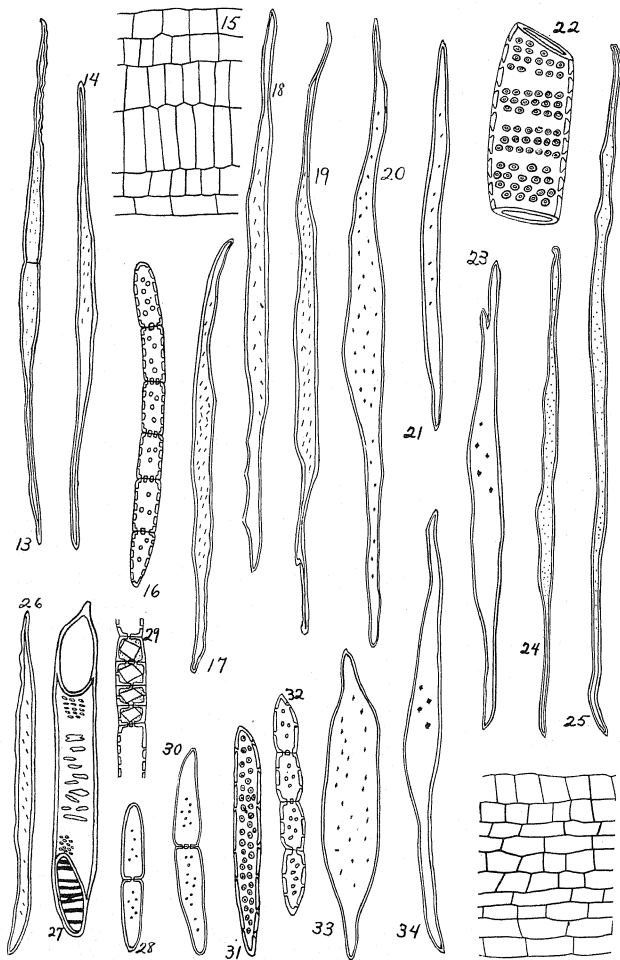




PLATE XXVIII

All outline drawings represent a magnification of 100 diameters.

FIGS. 13, 14.—Isolated wood fibers from wood of *Heliocarpus appendiculatus*.

FIG. 15.—Radial section of medullary ray of *H. appendiculatus*.

FIGS. 17, 19.—Isolated wood fibers from wood of *Apeiba aspera*.

FIGS. 18, 26.—Isolated wood fibers from wood of *H. popayanensis*.

FIG. 27.—Isolated vessel segment from wood of *H. appendiculatus*, showing both scalariform and porous perforations.

FIG. 29.—Portion of wood parenchyma strand, from wood of *H. popayanensis*, showing crystals.

FIG. 31.—Isolated fiber tracheid from wood of *H. popayanensis*.

FIG. 32.—Isolated wood parenchyma strand from wood of *H. popayanensis*.

FIGS. 20, 33.—Isolated wood fibers from wood of *Wercklea insignis*.

FIG. 30.—Isolated wood parenchyma strand from wood of *W. insignis*.

FIGS. 24, 25.—Isolated wood fibers from wood of *Pachira barrigon*.

FIG. 35.—Radial section of medullary ray of *P. barrigon*.

FIGS. 23, 34.—Isolated wood fibers from wood of *Cordia heterophylla*.

FIG. 28.—Isolated wood parenchyma strand from wood of *C. heterophylla*.

FIG. 21.—Isolated wood fibers from wood of *Cavanillesia platanifolia*.

FIG. 22.—Isolated vessel segment from wood of *Apeiba aspera*.

FIG. 16.—Isolated wood parenchyma strand from wood of *A. aspera*.

VARIATION IN SIZE AND FORM OF PYRUS SEROTINA

A. KIKUCHI

(WITH ONE FIGURE)

In connection with fertilizer experiments, the writer studied the effect of applications of different combinations of elements upon variations in the form of the fruits of the Chojuro variety of Japanese pear, *Pyrus serotina* Rehder. The effect of the growing season upon the size and form of the pears was also studied.

The fertilizer experiments under the plans shown in table I were started in 1909, at the Kanagawa Agricultural Experiment Station

TABLE I

Name given to plot	Element	Number of trees
NPK.....	Nitrogen, phosphorus, and potash	6
NP.....	Nitrogen and phosphorus	6
NK.....	Nitrogen and potash	6
PK.....	Phosphorus and potash	6
N.....	Nitrogen alone	6
P.....	Phosphorus alone	6
K.....	Potash alone	6
nf.....	No fertilizer (check)	3

near Yokohama. The writer took charge of the work in 1916. For three years previous to 1917 only the annual yield of three trees in each plot was recorded.

The experimental plots contained eight rows and forty-five trees. The trees in each plot and the rows of trees of adjacent plots were twelve feet apart. For three years previous to 1913, farm yard manure, soya bean cakes, wood ashes, sulphate of ammonia, superphosphate of lime, and sulphate of potash were applied to help the normal growth of the trees in all plots except the check. The quantities of fertilizer elements per acre given to the trees during these years are shown in table II.

Since 1913, commercial fertilizers alone were applied to the plots, and the trees in each plot were given either a single element or a combination, corresponding to the experimental plan shown in table I. Nitrogen in the form of sulphate of ammonia, phosphoric acid as phosphate of lime, and potash as sulphate of potash were applied. The annual rates of application per acre during the years 1913 to 1920 inclusive are given in table III.

The figures in tables II and III are computed in English measure, and therefore for the most part are fractional. The reason for this is that the Japanese unit was used for weight and area of land, and was

TABLE II
QUANTITY (IN POUNDS) OF FERTILIZERS RECEIVED BY PLOTS PREVIOUS TO 1913

Plot	1910			1911			1912		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
NPK.....	67	67	67	83	83	83	90	90	90
NP.....	67	67	17	83	83	17	90	90	17
NK.....	67	17	67	83	17	83	90	17	90
PK.....	17	67	67	17	83	83	17	90	90
N.....	67	17	17	83	17	17	90	17	17
P.....	17	67	17	17	83	17	17	90	17
K.....	17	17	67	17	17	83	17	17	90
nf.....									

then recalculated to the English unit. Applications of fertilizers were made annually in the early spring, and were well spaded into the ground. No cover crops were grown on any of the plots. The trees were annually cultivated twice in the dormant period, and three or four times in the active period.

The trees were trained on a horizontal trellis 5 feet 5 inches high, the so-called Japanese "tana," which is the most widely used system of training in Japanese pear culture (fig. 1). The trees were planted so closely that the roots of one tree often penetrated the soil of adjacent plots. Previous to 1916 this factor was not considered, but in the fall of 1916 the boundaries of each plot except the check were fixed by thick planks set into the ground to a depth of 2 feet 4 inches. Root pruning was given all trees at the same time. The boundaries of the check plot were boarded after the leaf-fall of 1917. At the same time each plot was trenched along the boundaries, and

roots which had grown through the cracks in the planks were cut. Subsequently the boundaries have been strictly determined every year.

Effect of fertilizers upon form of fruits

SHAW (2, 3, 4) reported his study on variation in the form of fruits as follows:

Some trees showed slight individuality in the amount of variability, and this may be correlated with size, the larger the apples the more variable. This is not true as between the different parts of the tree. As with size, some trees showed quite constant individuality in form of fruit, while others were variable. There seems to be no strong evidence that individuality in size and form is to be found in the tree. The apples from the upper south parts of the tree, which were larger, were also constantly the most flattened.

SHAW also observed a relation between variation in the form of fruits and the climatic condition. His statement is as follows:

There is a pretty constant relationship between the form of the apple and the temperature for a period following bloom; the cooler this period the more elongated the apple. An effort to delimit this period from the sixth to the sixteenth day following full bloom fits the observed fluctuation in form more closely than any other.

YOUNG (7) makes the following statement:

Some varieties remain quite constant in shape under different conditions, while others are much more plastic in this respect. The most frequent and conspicuous modification of form consists of the elongation of the axis of the fruit relative to the horizontal diameter. This is due to a diminished circulation of the sap caused by change in temperature, giving rise to an insufficient supply to provide for simultaneous development of the fleshy portion and elongation of the axis. Two or three weeks after blooming the form of the fruits becomes fixed and is not noticeably influenced by the moisture supply thereafter.

Variation in the form of the Ben Davis apple in Japan was studied in 1914. On a single tree the form was distinctly elongated in fruit produced on the terminal bud of the long, one-year old shoots, as compared with that on the spurs. The long shoots were divided into two groups in such a way that one group included those more than one foot long, and the other those less than one foot in length. The fruit from the former was always more elongated than that from the latter. The writer also made observations on the relation of the variation in form to the climatic condition in different localities. The

TABLE III
QUANTITY PER ACRE OF FERTILIZERS (IN POUNDS) RECEIVED BY PLOTS SUBSEQUENT TO 1913

Plot element	1913			1914			1915			1919 (16, 17, 18, and 19)				1920		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N
1. NPK.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200
2. NP.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200
3. NK.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200
4. PK.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200
5. N.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200
6. P.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200
7. K.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200
8. Check.....	100	100	100	133	133	133	167	167	167	183	183	183	200	200	200	200

TABLE IV
ORDER IN RANK OF ANNUAL AVERAGE VALUE OF FORM INDEX FOR EACH PLOT

Year	Order in rank							
	1	2	3	4	5	6	7	8
1918.....	nf (121.01)	K (121.71)	P (121.47)	NPK (121.17)	PK (121.10)	NK (120.77)	N (120.60)	NP (120.54)
1919.....	K (119.87)	PK (119.59)	NK (119.11)	nf (119.00)	P (118.36)	N (118.22)	NP (118.07)	NPK (118.06)
1920.....	K (121.01)	PK (120.76)	NK (120.60)	P (120.19)	nf (119.86)	NPK (119.59)	N (119.39)	NP (118.90)

summer temperature for three months showed a marked influence on the form of the fruit; that is, the higher the temperature the more flattened the apple.

The Chojuro pear was studied to determine whether there were more or less definite variations in form due to the application of different combinations of fertilizer elements. As stated, the trees were



FIG. 1.—Method of training pear trees on horizontal overhead trellis, the so-called Japanese "tana."

trained on horizontal trellises (fig. 1), and nearly all the fruit buds were produced on spurs. It therefore appears that there should be little or no variation in the form of fruits caused by their being on different parts of the tree, or by length of the bearing shoots. The Chojuro fruit is apple-shaped, rather round and regular, with a shallow cavity and basin.

The method employed to determine the form of the fruit was to record the largest transverse and longitudinal diameters of each individual fruit. These measurements were made with vernier cali-

pers reading to a tenth of a millimeter. The "form index" for the shape of each pear was obtained by dividing its transverse by its longitudinal diameter and multiplying the quotient by 100. The larger the form index the more flattened were the pears, and vice versa. The class value of the form index was made in ranges of 3, and the series of the class and the frequency of distribution were arranged as follows: class $\frac{101}{2}$ (99.5-102.4), $\frac{104}{5}$ (102.5-105.4), $\frac{107}{13}$ (105.5-108.4) (frequency of distribution is shown under each class value). The average value of the form index was calculated by the same method as used in determining the average weight of the fruits. The average values for the form index as obtained in 1918, 1919, and 1920, and the order in rank of the plots are given in table IV.

The range of variation in the form of the fruit caused by different combinations of fertilizer elements is not so wide as the range of variation in size. The extreme difference in the average index between the plots amounts to a trifle less than two in any one season, while between two individual trees in the same plot the difference was sometimes above two, although not often.

There appeared to be some relationship between the size of the fruit and its form. To obtain data the fruits from each of three trees of the Chojuro variety growing in different parts of the same orchard, and receiving fertilizers, were studied in 1919. The coefficient of correlation was calculated to determine the relationship between size and form for each of the three trees. The results were as follows:

Tree no.	Number of fruits observed	Coefficient of correlation
1.....	244	+0.009±0.068
2.....	152	+0.033±0.081
3.....	237	+0.007±0.065

The coefficient of correlation is positive for each, but so small as not to surpass its standard error in any of the three trees. The results show that there is no noticeable correlation between the form and the size of fruits produced on an individual tree. By comparing data pertaining to the effect of fertilizers upon size of fruits with table IV, it was determined that there is no definite relationship between the form of the fruit and its absolute size, even when fluctuation in size is caused by different combinations of fertilizer elements.

Table IV shows that the annual variation in the average index for each plot is in a constant direction. It suggests that some climatic condition favoring or opposing the development of fruits may cause definite variation in the form of fruits, although this apparently has no connection with their absolute size.

Data obtained from a study of the relation between the variation in the form of fruits and the climatic conditions prevailing during the growing season for each of three years are available. The average

TABLE V
AVERAGE MONTHLY TEMPERATURE (°C.) DURING
GROWING SEASON

Year	April	May	June	July	August
1918.....	12.8	16.9	21.1	26.7	26.6
1919.....	12.6	15.4	19.4	23.5	24.8
1920.....	12.7	16.9	20.5	25.9	25.1

TABLE VI
TOTAL MONTHLY PRECIPITATION (MM.) DURING
GROWING SEASON

Year	April	May	June	July	August
1918.....	166.4	131.7	128.7	55.0	154.9
1919.....	78.1	58.4	144.2	206.4	154.4
1920.....	173.7	285.9	252.2	241.2	522.8

temperature and the total precipitation from April to August are shown in tables V and VI. The average temperature for three years during each of the three weeks following full bloom is shown in table VII.

The Chojuro pear usually commences to blossom from the first to the fifteenth of April, and is harvested in late August or early September. The growing season of the fruit, therefore, in that part of Japan where the experiments were conducted, is not longer than five months. Table VI shows that the average temperature for each of the five months was lowest in 1919 and highest in 1918. The average value of the form index for each plot, without exception, was smallest in 1919 and largest in 1918. The annual difference in aver-

age temperature for each month was more noticeable in June, July, and August than in April and May. It is reasonable to suppose that there is a close correlation between the form of fruits and the average temperature during the growing season. No relationship was found between the monthly precipitation and the variation in the form of fruits.

Nothing of marked interest is indicated by a comparison of the average temperature during the three years for each of the three weeks following full bloom. In 1918 blooming continued from April 12 to 30, and the weekly temperatures were observed from the middle of the blooming date, or April 21. In 1919 the trees were in flower from April 3 to 15, and the observations were begun on the

TABLE VII
AVERAGE TEMPERATURE (°C.) FOR THREE WEEKS
FOLLOWING FULL BLOOM

Year	First week	Second week	Third week
1918.....	13.7	14.7	15.9
1919.....	12.5	14.5	13.1
1920.....	11.8	14.4	18.1

ninth. In 1920 the flowering continued from April 15 to 27, and the observations were started on April 21. From results presented in table VII it appears that there is little or no correlation between the form of fruits and the average temperature of the three weeks immediately following full bloom.

The time of maturity of the fruits in the different plots and the length of the growing season were noted. In 1918 the fruits in each plot matured earlier than in the other two years. All fruits were harvested on August 24 and 25. Their growing season was 126 days. The fruits produced in the check plot showed reddish brown color on August 15 or 16, and four or five days later those in the *K* plot began to mature. The *NP* and *N* plots matured last. There was little difference in the ripening dates of the other four plots. In 1919 all fruits were harvested on September 3 and 4. The growing season this year was 150 days. The fruits in the *K* plot were the earliest in their maturity, and those in the *NP* and *N* plots the latest. The ripening date of the check plot corresponded closely with that of the

K plot, but the amount of fruit harvested from the check plot was so small as to be of no value for comparison with other plots. In 1920 all fruits were picked on September 5 and 6. Their growing season was 138 days. The *K* plot showed the earliest, and the *NP* and *N* plots the latest maturity, as in the previous years. The trees in the check plot began to grow vigorously this year, which may account for the delayed maturity.

These results indicate that the form of fruits is influenced by the average temperature during the growing season, which hastens or delays the maturity of the fruits. It is also probable that variation in the form of fruits depends somewhat on the treatment of the tree, and that this treatment also affects the length of the growing season. The earlier the maturity the more flattened is the form of fruits.

Effect of growing season upon size and form of fruit

In the previous discussion a single tree or a certain plot was taken as a unit for the variation in the size and form of pears. The various stages of individual fruits, however, represent a life history of development. It is important, therefore, to observe the life history of the individual specimen in the study of variations in size and form.

Studies were made to determine whether there is a more or less definite period when pears make their chief increases in bulk and attain their fixed form, or whether increases in size and variation in form are both quite uniform and continuous throughout the growing season.

RIVIERE and BAILHACHE (1) found, in the case of pears, that there are more or less definite growth periods for the developing fruit. Winter and many fall varieties increase in size just previous to the time for picking, while summer and a few fall varieties make their chief increase in size about the middle of their growth.

WHITEHOUSE (6) reported, in his study of variation in apples during the growing season, that at least in the case of the Grimes there was a comparatively steady increase in size from the time of setting of the fruit until full maturity. There was no period of especially rapid growth, as reported by RIVIERE and BAILHACHE for pears. There was no especially marked increase in size of the Grimes

apple after the first of the fall rains in early September. WHITEHOUSE therefore stated that one would hardly expect to find such increases following a rain or irrigation during the growing season. He also reported in the case of the Tompkins King and the Fameuse varieties that the apples remained fairly constant in form, as compared with each other, throughout the growing season. In the Fameuse variety there was a tendency for apples to increase in transverse diameter relatively more rapidly than in longitudinal diameter; in other words, they became more oblate as the season progressed. This tendency was most pronounced in the early part of the growing season.

TAYLOR and DOWNING (5), in their studies on the irrigation of apple orchards, reported that Jonathan apples grew slowly from the time they were formed until July 15, completing less than 30 per cent of their total growth during the first half of the season. Irrigation during this period (before July 15) did not increase the size of the apples. Jonathan apples grew most rapidly during the period beginning about July 15 and ending about two weeks before picking time, when the rate of growth became considerably slower. Irrigation during this period of rapid growth had a decided effect in increasing the size of the apples, although it had almost no effect on the wood growth of the tree.

The writer's studies were carried on in 1918, 1919, and 1920. The first two years the observations were made on fruits on a tree grown in a plot treated with complete fertilizers, which was separated from the experimental fertilizer plot. The third year, trees no. 5 of both the *NPK* and *NP* plots were chosen for observation. The index for the form was obtained as previously outlined. The index for showing the size was deduced from the product of the transverse and longitudinal diameters of the fruit. The correlation between the real weight of the fruit and this index was studied. The coefficient of correlation was $+0.9188 \pm 0.0038$, calculated from 1616 matured fruits in 1918. From this result, the figure derived from the product of both diameter and length could be used with some confidence as a relative index to determine the size or weight of fruits, although the specific gravity of fruits might be different in the various stages of

growth. The intervals between measurements were from seven to eleven days, the irregularity being necessitated by weather conditions and other circumstances.

Originally 25 pears were selected for observation in 1918, and 24 of them were measured regularly. The other fruit dropped prematurely. In 1919, 40 pears were observed at the start, 30 of which were measured throughout the growing season. In 1920, 35 pears were originally selected on the tree in each of the two plots, but only 20 on tree no. 5 of the *NPK* plot and 23 on tree no. 5 of the *NP* plot were measured regularly.

Records were kept of the average temperature and the total precipitation throughout the growing season, so that it was possible to correlate these factors with any marked variation in rate of fruit growth or in the proportion in growth of both diameter and length. These data, together with the average indices of both the size and the form of the fruits in each period of measurement, are shown in tables VIII-XI.

Tables VIII and IX show that there was a period of especially rapid growth, comparable with that found for pears by RIVIERE and BAILHACHE. The fruit rapidly increased in size during this period as compared with other periods. It appeared in the interval between July 8 and 18 in 1918, and from June 28 to July 7 in 1919. In 1918 the maximum rate of growth took place during the period starting July 28 and ending August 6, or from the 99th to the 109th day following the date of full bloom. In 1919 the maximum rate of growth was during the period from July 18 to 28, or the interval between the 101st and the 112th day from full bloom.

The writer has applied the name "maximum period" to the period in the growing season during which the maximum rate of growth per day took place. When the fruit passed the maximum period the rate of increase in size became gradually slower until the harvesting time, in both 1918 and 1919. It is to be noted in both tables that there was not an especially marked increase in size following a period of rain.

With variation in form, the index increased continuously as the season progressed to the maximum period, or the period a little after it. The writer's studies gave much the same results as those obtained

TABLE VIII.

AVERAGE INDICES OF SIZE AND FORM IN EACH PERIOD OF MEASUREMENT; DAILY INCREASE IN SIZE AND INCREASE OR DECREASE OF FORM INDEX PER PERIOD, INCLUDING METEOROLOGICAL DATA (1918)

Data observed	May 28 June 7	June 8-13	June 19-29	June 30- July 7	July 8-18	July 19-29	July 28- August 6	August 7-16	August 17-24
Size in last of each period.....	640.07	887.93	1125.57	1375.29	1955.79	2599.14	3204.14	3844.59	4203.79
Increased rate in size per day.....	14.92	22.53	21.60	31.22	52.77	61.48	69.50	64.94	44.01
Form in last of each period.....	115.57	116.00	117.86	120.86	122.71	124.36	124.04	122.29	122.71
Variation in form in last of each period..	+2.64	+0.43	+1.86	+3.00	1.85	+1.05	+0.28	-2.35	+0.42
Average temperature (°C.).....	19.6	20.3	22.6	27.0	25.6	27.3	27.3	26.8	27.1
Total precipitation (mm.).....	24.5	30.9	82.9	14.6	40.4	34.2	37.3	0.0

TABLE IX

(1919)

Data observed	May 15-27 June 7	June 8-17	June 18-27	June 28- July 7	July 8-17	July 18-28	July 29- August 6	August 7-16	August 17-26
Size in last of each period*.....	839.83	1035.27	1321.17	1832.30	2538.43	3424.93	4132.17	4701.70	5300.50
Increased rate in size per day.....	17.07	19.54	28.59	51.11	70.61	80.59	78.58	65.95	51.78
Form in last of each period†.....	111.90	114.47	117.67	119.07	119.13	117.13	117.43	117.53	117.77
Variation in form in last of each period..	+2.60	+2.57	+3.20	+1.40	+0.06	-2.00	+0.30	+0.10	+0.24
Average temperature (°C.).....	18.5	18.0	21.1	22.4	22.6	24.8	24.9	24.3	23.7
Total precipitation (mm.).....	76.3	39.1	28.2	31.3	12.6	8.2	210.5	97.7	1.3

* 390.80 on May 14.

† 204.87 on May 14.

by WHITEHOUSE in his studies on apples. When the fruit passed the maximum period some irregularities took place in the direction of variation in form. These results indicate that the maximum period is the most important time for the observations on the variation in the pear during its growing season.

A comparison of the data presented in tables X and XI is of interest. The observations were made after June 23 in 1920. The fruits on tree no. 5 of the *NP* plot averaged 87 per cent as large as those on tree no. 5 of the *NPK* plot at the beginning of the measurements. At harvesting time on September 6, the former was 41 per cent the size of the latter. The maximum period was reached one period earlier in the *NP* than in the *NPK* plot. In the former case the maximum period appeared in the interval between the 83d and the 92d day from the date of full bloom. In the *NPK* plot the period of most marked increase of the daily rate of growth coincided with the maximum period. There was no period of rapid growth in the *NP* plot, but the rate of growth became low after early August. The rate of growth was also slow in the season previous to the maximum period in the *NP* as compared with the *NPK* plot. With regard to variation in form, the same results were obtained as in 1918 and 1919. The data presented in the four preceding tables, although showing some irregularities, indicate that the higher the temperature the more flattened the fruits, especially in the weeks previous to the maximum period.

Summary and conclusions

1. There is no noticeable correlation between the form and the size of fruits produced on an individual tree of the Chojuro variety of *Pyrus serotina*.
2. There is a close relationship between the form of fruits and the length of the growing season.
3. The form of fruits is influenced by the average temperature during the growing season, which hastens or delays their maturity.
4. No relationship, however, is observed between the monthly precipitation and the variation in the form of fruits.
5. It is probable that variation in form of fruits depends somewhat on the treatment which affects the length of growing season or ripening period. The earlier the maturity the more flattened is the form.

TABLE X
NPK PLOT (1920)

Data observed	June 23	June 24- July 1	July 2-11	July 12-21	July 22- August 1	August 2-11	August 12-16	August 17-26	August 27- September 6
Size in last of each period.....	859.20	1013.05	1338.25	1833.95	2605.20	3230.30	3427.80	3813.65	4141.70
Increased rate in size per day.....	10.23	32.52	49.57	70.11	62.51	39.59	38.59	20.82
Form in last of each period.....	108.90	109.85	112.95	116.00	117.70	118.55	119.95	119.85	119.15
Variation in form in last of each period.....	+0.95	+3.10	+3.05	+1.70	+0.85	+1.40	-0.10	-0.70
Average temperature (°C.).....	21.3	25.9	26.3	25.9	24.6	25.7	25.5	24.5
Total precipitation (mm.).....	29.4	35.8	0.2	205.2	344.3	26.4	147.4	139.6

TABLE XI
NP PLOT (1920)

Data observed	June 23	June 24- July 1	July 2-11	July 12-21	July 22- August 1	August 2-11	August 12-16	August 17-26	August 27- September 6
Size in last of each period.....	758.00	936.52	1185.00	1466.00	1737.26	1897.91	1934.43	1994.06	2061.22
Increased rate in size per day.....	22.32	24.85	28.10	24.66	16.07	7.30	6.05	6.02
Form in last of each period.....	112.22	113.26	117.52	119.17	120.70	119.78	119.91	120.26	118.65
Variation in form in last of each period.....	+1.04	+4.26	+1.65	+1.53	-0.92	+0.13	+0.35	-1.01
Average temperature (°C.).....	21.3	25.9	26.3	25.9	24.6	25.7	25.5	24.5
Total precipitation (mm.).....	29.4	35.8	2.0	205.2	344.3	26.4	147.4	139.6

6. The term "maximum period" has been applied to the period in the growing season during which the maximum rate of growth per day takes place.

7. The maximum period of Chojuro pears, in the Kanagawa Agricultural Experiment Station plots, appears about the 100th day from the date of full bloom, when the fruit develops under normal conditions.

8. Generally the form of the pear becomes more and more oblate in the season previous to the maximum period, and there is no definite direction of variation in the form in the season following this maximum period.

9. The writer does not consider the data conclusive; nevertheless they indicate that when relatively high temperatures prevail during the growing season previous to the maximum period, the form of fruits is more flattened than when lower temperatures exist.

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CONDITIONS FOR GERMINATION OF SPORES OF ONOCLEA SENSIBILIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 336

CONSTANCE E. HARTT

(WITH PLATE XXIX, AND ONE FIGURE)

Conflicting accounts have been given by various workers concerning the conditions for germination of fern spores. No adequate explanations have been made of these discrepancies. As the results of each investigator are usually consistent, it seems probable that some undescribed conditions of experimentation vitally affect the work. The factors which have been noted as influencing germination are temperature, intensity and refrangibility of light, culture media, and age of spores, and it is to be regretted that these data are often insufficient in the literature.

Historical

No mention is made of temperature by KAULFUSS, LESZYC-SUMINSKI, THURET, MERCKLIN, WIGAND, or HOFMEISTER, except that MERCKLIN considered a certain temperature necessary. BORODIN (3), using water cultures of *Aspidium spinulosum*, found that the spores exposed to full lamplight germinated before those exposed to lamplight from which the heat rays were absorbed by interposing a vessel filled with water. SCHELTING (17) investigated the influence of temperature on germination, and found that *Aneimia Phyllitidis* always germinated earlier at 30° C. than at 18° C., and with further development showed that at the higher temperature the cells divide more rapidly but grow in length more slowly. He further stated that higher temperatures will cause germination in darkness, a statement which WORONEW contradicted. WORONEW (20) obtained germination in the dark on a nutrient solution at 28° C., but claimed that germination was slower than at a lower temperature in the light.

FISCHER (8), however, stated that spores of *Polypodium vulgare* germinated better in the dark at 25° C. than in daylight at room temperature, but no mention is made of the culture medium used. According to SCHULTZ (18), other stimuli are not able to replace light, except in the case of *Ceratopteris thalictroides*, in which high temperature may. HEALD (9), working with *Alsophila Loddigesii* and *C. thalictroides*, found that a temperature of 32° C. will furnish the necessary stimulus for germination in the dark. He stated that germination depends upon certain chemical changes in foodstuffs stored in the spores; these changes may be caused by light or a temperature of 32° C. KNY (12), working with *C. thalictroides*, found that favorable temperature conditions are necessary, but did not state the temperature specifically.

Intensity of light is mentioned by several investigators. LIFE (14) found that germination is best in light of medium intensity. BECK (2) and KNY (11) both found that light of "sufficient intensity" is a necessary condition for germination. According to NAGAI (15), strong light is injurious to the germination of spores of *Ceratopteris thalictroides*, and according to WORONEW bright light retards growth. The effect of weak light was discussed by HOFMEISTER (10): "In those species where considerable elongation of the lowest cell occasionally takes place, it seems to be caused by deficiency in light."

Considerable work has been done to ascertain whether or not spores will germinate in absolute darkness, the varied results of which are given in table I.

SCHELTING found that spores germinate well in the dark, and form a small amount of chlorophyll which is lacking in the spore, but LAAGE claimed that chlorophyll is not formed during germination in the dark. He obtained no stages in germination in the dark more advanced than the bursting of the exine.

Results on the effect of different qualities of light are given by BORODIN, SCHULZ, HEALD, and BURGERSTEIN (5). BORODIN, having found to his satisfaction that light is a condition necessary for germination, proceeded to determine which light rays are the most effective. Using water cultures of *Aspidium spinulosum*, *Ancimia Phyllioides*, and *Allosorus sagittatus*, he was able to germinate spores in yellow light but not in blue. He isolated yellow rays by passing

TABLE I
GERMINATION IN THE DARK

Investigator	Fern	Result	Temperature (C.)	Medium
Borodin (1868).....	<i>Aspidium spinulosum</i> var. <i>foeniculi</i>	o	Neva River water
	<i>Aspidium molle</i>	o	" " "
	<i>Aneimia Phyllitidis</i> var. <i>longifolia</i>	o	" " "
	<i>Allosorus sagittatus</i>	o	" " "
	<i>Asplenium alatum</i>	o	" " "
	<i>A. (Displasium) lasiopteris</i>	o	" " "
	<i>Polypodium repens</i>	o	" " "
Goepfert (1869)....	<i>Phegopteris effusa</i>	o	" " "
Schmidt (1870).....	<i>Osmunda</i> sp.	+
	<i>Aspidium Filix-mas</i>	o
Kny (1872).....	<i>Aspidium violaceus</i>	o
	<i>Osmunda</i> sp.	o
Schelting (1875)....	<i>Aspidium Filix-mas</i>	+	Soil and water
	<i>Aspidium falcatum</i>	+	" " "
	<i>Aneimia Phyllitidis</i> var. <i>longifolia</i>	+	30° and 18°
	<i>Pteris aquilina</i>	+	" " "
	<i>Scolopendrium vulgare</i>	o
Beck (1878).....	<i>Alsophila Loddigesii</i>	o	19-21°	Filter paper, pieces of flower pots, sterile earth in Petri dishes
Heald.....	<i>Ceratopteris thalictroides</i>	o	19-21°	
	<i>Ceratopteris thalictroides</i>	+	32°	
Burgerstein (1900)..	<i>Osmunda</i> sp.	o	Haide erde und peat
Schulz (1902).....	<i>Pteris</i> (3 spp.)	o
	<i>Aspidium falcatum</i>	o
	<i>Aspidium Sieboldii</i>	o
	<i>Asplenium Filix-foemina</i>	o
	<i>Polypodium aureum</i>	o
	<i>Didymochlaena lunulata</i>	o
	<i>Doodia caudata</i>	o
	<i>Scolopendrium officinale</i>	o
	<i>Gymnogramme chrysophylla</i>	o
	<i>Aneimia Phyllitidis</i>	o
	<i>Alsophila elegans</i>	o
	<i>Alsophila australis</i>	o
	<i>Ceratopteris thalictroides</i>	+
	<i>Osmunda regalis</i>	+
	<i>Pteris aquilina</i>	+
	<i>Scolopendrium officinarum</i>	+	Knop's solution
	<i>Aspidium Filix-mas</i>	+	" "
Laage (1906).....	<i>Polypodium Dryopteris</i>	+	" "
	<i>Pteris cretica</i>	+	" "
	<i>Aspidium aculeatum</i>	+	" "
	<i>Aspidium spinulosum</i>	+	19°-21° and 30°	" "
	<i>Balanium antarcticum</i>	+	" "
	<i>Asplenium lucidum</i>	o	" "
	<i>Alsophila australis</i>	o	" "
	<i>Polypodium aureum</i>	o	" "
	<i>Dicksonia apiifolia</i>	o
	<i>Alsophila pruinata</i>	o
Life (1907).....	<i>Aneimia Phyllitidis</i>	o	30°-33°	Leaf mold
	<i>Gymnogramme calomelanos Mertensii</i>	+	30°-33°	0.5 per cent Knop's solution
	<i>Gymnogramme calomelanos Mertensii</i>	o	30°-33°	Leaf mold
	<i>Gymnogramme calomelanos Mertensii</i>	o	Room	0.5 per cent Knop's solution in agar-agar
	<i>Gymnogramme calomelanos Mertensii</i>	o	15°, 25°-27°
Nagai (1914).....	<i>Adiantum tenerum</i>	o
	<i>Adiantum fulvum</i>	o	"	"
	<i>Adiantum peruvianum</i>	o	"	"
	<i>Adiantum macrophyllum</i>	o	"	"
	<i>Asplenium bulbiferum</i>	o	"	"
	<i>Asplenium Belangerii</i>	o	"	"
	<i>Woodwardia radicans</i>	o	"	"
	<i>Nephrolepis davallioides</i>	o	"	"
	<i>Nephrolepis exaltata</i>	o	"	"
	<i>Cibotium Schiedeii</i>	o	"	"
	<i>Balanium antarcticum</i>	o	"	"
	<i>Ceratopteris thalictroides</i>	+	"	"
	<i>Aspidium Filix-mas</i>	o	Organic and inorganic nutrient solution
	<i>Aspidium fulvum</i>

light through a solution of potassium dichromate; for blue rays, he used ammoniated copper oxide. He concluded that germination is due to the less refrangible rays, those of higher refrangibility acting in a manner similar to complete darkness, or in any case their effect being much weaker than the yellow rays. SCHULZ found that moss and fern spores germinate in the yellow and blue halves of the spectrum, as he obtained germination of several species of fern spores in white and red rays in twenty days, and in blue in thirty days. HEALD, working with *Marchantia* spores, found that they do not germinate in darkness, and that blue rays have the same effect as absolute darkness. BURGERSTEIN, using twenty-four species of ferns, found that under the influence of blue rays the prothallia were formed several days to weeks later, never earlier, than with the less refrangible rays. No prothallia of *Gymnogramme sulfurea* were formed in blue light.

Data on the age of spores show varied results. TREBOUX (19) stated that old moss spores often show slight viability in darkness, and will germinate in light only when provided with glucose. Miss ROGERS (16), working with *Lygodium palmatum*, found that spores planted at the end of three months produced less vigorous prothallia than those planted directly after collection. She found that each successive planting produced prothallia of decreasing vigor, as shown by their smaller size and slower growth. The rate and percentage of germination were decreased. Practically all the spores planted directly after collection germinated, but of those which were kept seven months, 50 per cent began to crack only after twelve days. CAMPBELL (6) found that the spores of *Onoclea Struthiopteris* contain considerable chlorophyll, and germinate in three to five days. They retain their viability several months after being gathered.

Several examples of the longevity of fern spores are found in the literature. COLEBY and DRURY (7) reported the germination of spores of *Dicksonia* twenty years old, and of spores of *Gymnogramme aurea* which must have been dormant a long time. According to WRIGHT (21), spores of *Cheilanthes mysurensis*, which were collected in October, 1899, and preserved in a herbarium, were sown in March, 1908, and produced healthy plants. In 1910 (1) a report appeared of

spores of *Dicksonia antarctica* which produced a large number of healthy plants after being in a herbarium twenty-two years. LAAGE, however, was unable to germinate six-year old spores of *Aspidium Filix-mas*, *Aspidium angulare*, *Alsophila procera*, or *Dicksonia Wendlandi*.

According to KNY (11), old spores of *Osmunda regalis* show an appearance deviating very much from that of fresh spores. Their content is shrunk. The oily drops have come together in one or more larger drops. Because of thinness of exine and rich chlorophyll content, their germination capability is of short duration.

While these investigators have shown that age is a factor in decreasing the germination capability of spores, LIFE has shown, in one case at least, that age is important in causing germination. *Alsophila australis* spores need a year's rest period between collection and germination.

Materials and methods

It is apparent that the germination of spores should be studied from the point of view of limiting factors. The experiments recorded here deal with the following factors: intensity of light, effectiveness of rays of different refrangibility, continuous light, temperature, and age of spore. The culture medium in all cases was distilled water. One species of fern was used, *Onoclea sensibilis*. In all experiments efforts were made to keep all conditions uniform except one factor.

Varying intensities of light were obtained under the following conditions: (1) full sunlight, all day; (2) intense sunlight, south window with sunlight for six hours in middle of day; (3) moderate sunlight, east window with sunlight four hours in morning and diffused light rest of day; (4) intense diffused light, north window; (5) moderate diffused light, laboratory table 18 feet from north window; (6) weak diffused light; (7) absolute darkness, as indicated by photographic tests. Constant temperature was maintained in water baths with thermostat control.

Rays of different refrangibility were obtained by using red and blue colored glass prepared at the Desert Botanical Laboratory at Tucson, Arizona. The red glass admitted only red rays of the spectrum, while the blue admitted green, blue, and violet rays, when

tested with a spectroscope. Cultures were kept under red and blue glass in north, east, and south windows, and in continuous light. Continuous light of constant intensity was obtained by using an electric bulb in a light-proof, wooden box lined with white paper.

To ascertain the relation between viability and age of spores, experiments were made with fresh material collected when shedding, and some which had been stored in a refrigerator at 12° – 13° C., and some stored at room temperature for periods varying from four weeks to ten months. The spores of *Onoclea sensibilis* are formed during the summer and fall, and remain over winter in the sporangia in apparently mature condition. They are shed in the open, in Massachusetts, during April and the first part of May. These large, oval spores vary in size from 58 to $73 \mu \times 67$ to 76μ , and contain considerable chlorophyll. The spore of *O. sensibilis* is similar to that of *O. Struthiopteris*, as described by CAMPBELL, in having three thin spore coats through which the single large nucleus may easily be seen. KNY also found that the nucleus of the spores of *Osmunda regalis* is often visible in an uninjured spore. When this is not possible, a slight pressure on the cover slip will cause the spore to emerge from its outer coat, thus facilitating observation of the earlier stages of germination which take place before the rupture of the exine. The first step is the division of the nucleus. The resulting two daughter nuclei may frequently be seen without the removal of the exine, before the dividing wall has formed. The splitting of the exine normally takes place after the formation of the first wall. The succeeding stages are of the usual polypod type (figs. 1–7). Germination in favorable conditions in the laboratory requires two to three days, but has been obtained in twenty-six hours. In April, 1922, the germination period in the open, when the temperature varied 5° – 16° C., was one week, with a very low percentage of germination.

Observations and results

EFFECT OF DIFFERENT LIGHT INTENSITIES

As these experiments, lasting from February 10 to April 18, gave consistent results, an account of a typical set will be given. On April 10, spores were planted on distilled water within two to four

hours of collection. The temperature ranged from 21° to 23° C. The spores receiving intense diffused light always germinated most rapidly and in greatest numbers. Those receiving moderate and weak diffused light had a longer germination period than those in intense diffused light, while those in absolute darkness germinated even more slowly. In general, those in absolute darkness and those in weak diffused light developed longer filaments than those receiving intense diffused light, as a result of which the chlorophyll of the former was less dense (figs. 8-10), but some germinated normally. Those in moderate sunlight germinated more slowly and less abundantly. Very few germinated in intense sunlight, many losing their

TABLE II

RELATIVE STANDING AFTER 29-34 HOURS

1. Full sunlight.....	0
2. Intense sunlight.....	1 out of many
3. Moderate sunlight.....	13 out of 100
4. Intense diffused light.....	24 out of 52
(in other experiments a larger proportion germinated)	
5. Moderate diffused light.....	21 out of 59
6. Weak diffused light.....	10 out of 36
7. Absolute darkness.....	6 out of 30

chlorophyll (fig. 11). There was no germination in full sunlight, most of the spores losing their chlorophyll and dying. The results of this experiment are shown in table II and text fig. 1.

The question of chlorophyll formation in the dark is a difficult one. Resting spores contain chlorophyll but in no definite shape, while filaments produced in the light or dark contain chloroplasts; therefore some change takes place, the nature of which was not studied.

EFFECT OF TEMPERATURE

Data on the effect of temperature on germination were obtained in intense diffused light, absolute darkness, and continuous light. The experiments with continuous light are discussed later.

The object of these experiments was to determine the minimum, optimum, and maximum temperatures at which germination takes place in intense diffused light and in absolute darkness. Experiments

conducted by Miss STOKEY of Mount Holyoke College, assisted by a student, and others by the writer, lead to the supposition that the minimum temperature for germination in intense diffused light is around 15°C ., the optimum between 27° and 33° , and the maximum between 35° and 40° . These experiments were conducted without absolute temperature regulation.

Using electrically controlled water baths, a series of experiments was conducted in intense diffused light and absolute darkness, with the results as shown in table III.

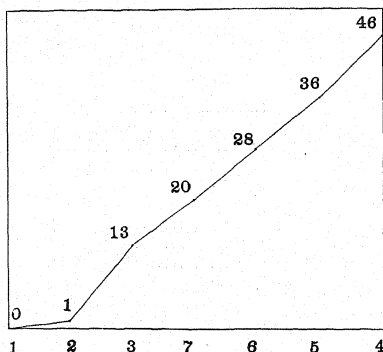


FIG. 1

That 23 per cent germination was obtained at 17°C . seems not to be in accord with the results at 16° and 18° . It is possible that a slight variation in the temperature occurred during the night. The table shows that germination was first obtained at 16° in the light, and about 24° in the dark; that the highest percentage of germination in the light was obtained at 28° , and in the dark at 28° – 29° ; that the maximum temperature for germination was 34° in the light and 33° in the dark.

Not only does temperature affect the percentage of germination, but also the character. Prothallia produced at 16° – 28° in the light

were similar as to size of cells and distribution of chlorophyll. Those at 28° were further advanced in three days than those at the lower temperatures. Those at 29°-31° showed abnormal growth; for example, twinning, bent prothallia, rounded cells, and chlorophyll concentrated in the tip cell (figs. 12, 13). Some ungerminated spores at 31°-34° contained large fatty globules, resembling old spores. Spores kept at 35°-40° showed diminution in amount of chlorophyll.

TABLE III
GERMINATION IN THREE DAYS

Temperature °C.	Light	Dark
12.....	o	o
13.....	o	o
14.....	o	o
15.....	o	o
16.....	2 (10 per cent in 4 days)	o
17.....	23 per cent	o
18.....	14 per cent	o (1 doubtful)
21.....	20 per cent	o
24.....	Many	1
27.....	Many	6-7 per cent
28.....	100 per cent (normal)	95 per cent
29.....	85-90 per cent (abnormal)	95 per cent
30.....	90 per cent (abnormal)	30 per cent
31.....	30 per cent	30 per cent
32.....	Abnormal	16-20 per cent
33.....	Very few	Very few
34.....	Very few	o
35.....	o	o
36.....	o	o
40.....	o	o

Spores were kept at 35° for three days and then at room temperature. In three days a few had germinated, showing that they were not killed by 35° C. Spores which had been kept at 36° for three days were placed at room temperature and four germinated in two days; 40° was found to kill the spores.

It is probable that germination depends upon the activity of certain enzymes. Possibly their reactions toward temperature may be explained by certain facts true of other physiological reactions; namely, that at normal temperature their actions are coordinated, but just below the maximum temperature there is more or less loss of self-regulation and coordination, which stops growth without

killing the organism. If the unfavorable condition does not last too long, activities become normal again with returning normal conditions; but if the temperature rises too high, cell death occurs.

EFFECT OF RAYS OF DIFFERENT REFRANGIBILITY

The spores kept under red and blue glass in intense sunlight did not germinate. As with full light, these experiments showed that intense diffused light is more favorable for germination than partial direct sunlight. In twenty-nine hours, half of those receiving the red rays in intense diffused light were beginning to germinate, whereas only one-fifth of those receiving the more refrangible rays were germinating. This experiment was repeated several times, and every time those under the red glass in intense diffused light germinated the most successfully. These results, therefore, agree with those of BORODIN and HEALD, in that the less refrangible rays of the spectrum were found to be the most effective in promoting germination. The results differ in that the writer obtained germination with the more refrangible rays, while they did not. They experimented with spores containing little or no chlorophyll, however, while those of *Onoclea sensibilis* contain a large amount.

EFFECT OF CONTINUOUS ARTIFICIAL LIGHT

Almost all the spores kept at 31° and receiving full light germinated in forty-two hours, the exine splitting and green protruding. The primary rhizoid of some spores had begun to develop. A very few of those in the dark were cracking, many showing partial or complete loss of chlorophyll. Almost all germinated in red light, while only half germinated in blue. Two of those at 37° receiving full light germinated; the others were less green than normal, the chlorophyll having shrunk to the centers of the spores. Of about two hundred in the dark, nine or ten germinated. All the spores were greener than those in the light, but not as green as normal. A few of those receiving red rays germinated, but were not normally green. Those receiving blue rays showed results similar to those in absolute darkness. The temperatures mentioned are doubtful, as there was no adequate means of control. Subsequent work with more accurate control makes it seem highly improbable that any germination took place at 37° C.

VIABILITY OF SPORES OF DIFFERENT AGES

These experiments were undertaken because the best results obtained in absolute darkness were in February and March, while in the last part of April and the first part of May the percentage of germination was low, and in the last part of May there was no germination at all. The spores of the first experiments were used a few hours after collection, whereas those used later had been stored four to six weeks at 12° – 13° . It would appear from this that fresh material is the best. To prove this, an attempt was made in the last part of May to find fresh material in the open. After a successful search it was found that material collected in the last part of May will not germinate in darkness in the usual time. The majority germinated in eight days, a few with long filaments of only one cell, but the greater number with two normal cells. These results suggest that germination is influenced by the age of spores.

Spores were collected near South Hadley, Massachusetts, in May, 1923, and stored about a month at 12° – 13° ; afterwards at room temperature. At the end of March, 1924, many of these germinated in light in five days, and a very few in the dark in ten days.

Spores were collected near Smith, Indiana, July 7, 1923, and stored at room temperature. From January 4 to 10, 1924, a few germinated in light and in the dark, but from March 28 to April 7 there was no germination either in light or dark, and there was no visible chlorophyll in the spores. Spores were sown May 8 and kept in intense diffused light at 28° – 30° . By May 13 five spores had germinated, while most of the others were brown or almost colorless. It was evident from the position of these five spores that they had come from a single sporangium which had afforded them protection.

Spores collected December 17, 1923, at Greensboro, North Carolina, and stored at room temperature, were planted March 28. After five days in the light many were two or three celled, while after ten days in the dark only three germinated, forming long, single celled prothallia with the chlorophyll in the tip. These results suggest that spores retain their germination ability in light longer than in the dark.

Little work has been done to determine the chemical difference

between mature and old spores. Fresh spores contain fat (but not in conspicuous globules), a little protein, and no starch. They are richly supplied with chlorophyll. The older a spore becomes the less chlorophyll it contains, until none is left. Old spores contain many conspicuous fatty droplets of various sizes (fig. 14). Similar globules may be obtained by heating fresh spores in 95 per cent alcohol on the water bath.

While these factors have been treated separately, it must not be supposed that they can be absolutely divorced. For instance, old spores will germinate at higher temperatures but not at medium or low, and in intense diffused light spores will germinate at temperatures lower than in absolute darkness. Since *Onoclea sensibilis* spores germinate over a wide range of light and temperature intensities, but lose their viability in a comparatively short time, it is suggested that age may be the limiting factor.

Summary

1. These experiments deal with the conditions for germination of spores of *Onoclea sensibilis*.

2. Spores germinate best in diffused light, and intense diffused light is better than moderate or weak.

3. Sunlight is unfavorable to germination, and intense, long continued sunlight destroys chlorophyll.

4. Spores germinate in absolute darkness.

5. The highest percentage of germination in continuous light was obtained at 31° C.

6. The minimum temperature for germination in intense diffused light is 16°, the optimum 28°, and the maximum 34°; 40° kills the spores.

7. The minimum temperature for germination in absolute darkness is 24°, the optimum 28°-29°, and the maximum 33°.

8. The less refrangible rays of the spectrum are the most effective in causing germination, whether intermittent or continuous.

9. Viability in light and darkness depends upon age of spores. Spores retain the ability to germinate in light longer than the ability to germinate in the dark.

10. Old spores differ from fresh ones in that they contain less chlorophyll, and the fat is present in more conspicuous globules.

11. Since *O. sensibilis* spores germinate over a wide range of light and temperature intensities, but lose their viability in a comparatively short time, it is suggested that age may be the limiting factor.

12. It is suggested that the conflicting accounts of previous investigators have been caused by some undescribed conditions of experimentation, such as variations in temperature, light intensity, types of media, or age of spores.

The writer wishes to express deep gratitude to Dr. ALMA G. STOKEY of Mount Holyoke College, and to Dr. CHARLES A. SHULL of the University of Chicago, for suggestions, help, and criticism of these experiments.

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EXPLANATION OF PLATE XXIX

All figures except fig. 14 were drawn with a camera lucida $\times 340$.

FIGS. 1-7.—Stages of normal germination.

FIGS. 8-14.—Abnormal forms.

FIG. 8.—After 72 hours in weak diffused light.

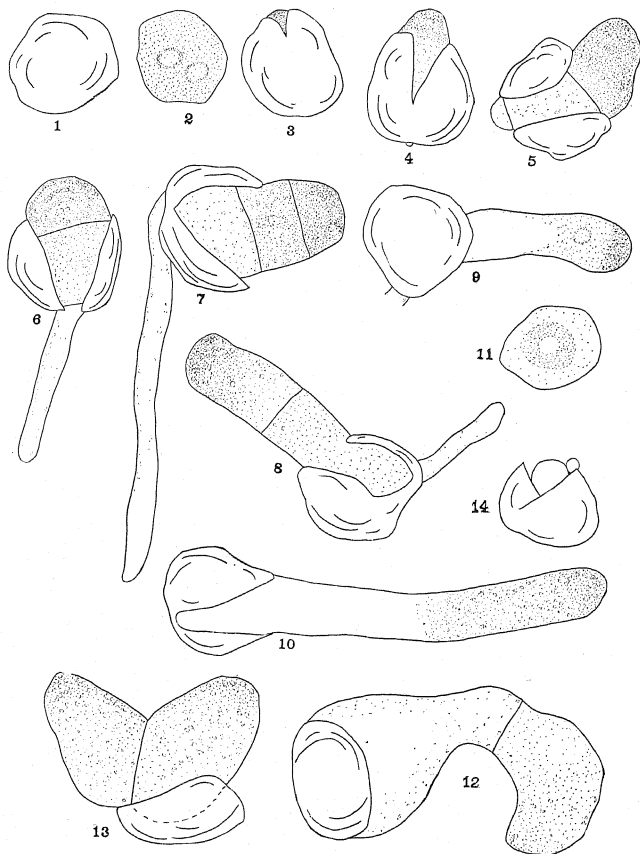
FIG. 9.—After 66 hours in absolute darkness.

FIG. 10.—After 72 hours in absolute darkness.

FIG. 11.—Diminution of chlorophyll content, as in old spores, or those exposed to intense sunlight or to temperatures of 31° - 40° C.

FIGS. 12, 13.—Bent prothallium and twins formed at 29° - 31° C.

FIG. 14.—Crushed spore with fatty globules.



A NEW SPECIMEN OF LEPIDOSTROBUS FOLIACEUS
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 337

JUAN D. CAMPOS

(WITH PLATES XXX-XXXII)

The series of sections which forms the principal basis of this paper was sent by Mr. LOMAX of Lancashire, England. As the type specimen (C. N. 1614, WILLIAMSON 8) on which MASLEN founded this species was incompletely preserved, only a provisional diagnosis (4) was given. With better material on hand, excellently preserved and prepared, it seemed wise to direct attention to an intensive study of the structure of this species so that a fuller description of it could be given.

The material studied consists of a series of twelve transverse sections of the cone, slides G 8-19. According to LOMAX, it is "the most perfect specimen of a lycopodiaceous cone that has yet been discovered." A comparison of this with those described and figured in literature will justify the claim. G 8 is cut through the apex and G 19 through the base of the cone. The length of the cone was originally 2 inches, or 5.1 cm., while measurements of the middle sections give an average of 2.4 cm. in diameter. The Halifax Hard Bed at Huddersfield, Yorkshire, England, from which the specimens were collected, is a coal seam of the Lower Coal Measure Formation in the Gannister Horizon.

Cone

The diagrammatic reconstruction shown in fig. 9 will give an idea of the general form and structure of the cone. This reconstruction was based upon the twelve transverse sections. Each section was carefully traced on a blank sheet with camera lucida. One of these tracings is shown in fig. 1. After measuring each part of the cone traversed by the dotted line ($x-y$) across the longer diameter of the section, it was plotted on a graph sheet. A general survey of all the slides suggested a bent axis, for some of the sporangia were

cut transversely on one side of the axis and tangentially on the other. Only a bent axis as shown will fit the situation, taking for granted that the sections were cut parallel to each other from the block. As there were no longitudinal sections available from this same material, the diagrammatic longitudinal section of a sporophyll attached to the axis (fig. 14), together with those figured by WILLIAMSON and MASLEN, helped in roughly mapping out the form and relation of the various parts. As shown by the reconstruction, the general form is ovoid, measuring a little over 5.1 cm. in length and 2.4 cm. in diameter through the widest portion.

Cone axis

The axis is in excellent condition for an intensive study of the internal structure throughout its entire length. For this reason it would offer a good material for finding, as MASLEN suggested, "what variations in structure, if any, take place in different regions in the same cone."

The cone axis consists of a definite pith, a rather broad primary xylem bordered with protoxylem cells bulging out into points in several places around the xylem cylinder, a 1-2-celled meristematic region, and a narrow undifferentiated cortex (primary) containing sporophyll traces (fig. 2). Table I shows the dimensions secured from the slides, G 19 representing the lowest section, G 14 a middle section, and G 9 the second to the top slide. From this table it will be seen that: (1) the axis is long and slender, tapering to a point toward the top; (2) the pith is smaller at the bottom than at the top; (3) the primary xylem is thicker at the bottom than at the top; (4) the cortex maintains the same thickness throughout; (5) the meristematic region is very narrow, and maintains the same thickness throughout; (6) the protoxylem points number from 7 to 15 on each section, there being less in the top sections; (7) the sporophyll traces on each section are the same in number throughout the length of the axis.

Fig. 3 shows three stages in the separation of the sporophyll trace bundles from the peripheral protoxylem points. At *a* is a protoxylem point just beginning to separate, at *b* is another bundle with one or two meristematic cells inserted between the sporophyll

trace bundle and the xylem cells inside, and at *c* is a large one wholly separated from the xylem cylinder, but still imbedded in the cortex. On account of the absence of longitudinal sections it is impossible to give an account of the course of the sporophyll trace bundles through the cortex.

Sporophyll

Fig. 14 shows a reconstruction of the sporophyll bearing a sporangium on its adaxial surface. This reconstruction was based upon a series of sections (fig. 10) of a sporangium running through slides G 11-19. As in the former reconstruction, the whole was plotted on a graph sheet.

TABLE I
DIMENSION AND NUMBER OF PARTS OF CONE AXIS

Parts	Base G 19	Middle G 14	Top G 9
	mm.	mm.	mm.
Diameter of axis.....	1.28	0.95	0.53
Diameter of pith.....	0.25	0.26	0.30
Thickness of X ²	0.29	0.26	0.24
Thickness of meristem.....	0.002	0.002	0.002
Thickness of cortex.....	0.08	0.09	0.09
No. of protoxylem points.....	12	15	7
No. of sporophyll traces.....	9	9	9

As the sporophyll trace bundles leave the axis to form the pedicel, they carry with them an envelope of cortical parenchyma and assume a peculiar triangular shape (fig. 6 to fig. 10 *a*). The one corner turned toward the axis is blunt, while the outer two are sharper pointed. In the better preserved specimens may be recognized a row of epidermal cells. The greater part of the pedicel is taken up with thick walled parenchyma, leaving only a small area in the center for thinner walled parenchyma surrounding a simple bundle. This bundle consists of the same number (30-70) of elements as when they were in the cortex, and measures 11 mm. across. It has no definite protoxylem cells. In most of the pedicels there is a space on the dorsal side of the bundle which corresponds with MASLEN'S "phloem region." The thin walled parenchyma surrounding the bundle may be taken as a continuation of the meristematic region of the axis. In this connection may be mentioned the presence in some of the pedicels of a similar space on the ventral side of the bundle. Fig. 4

shows this space as the result of the breaking down of the fine parenchymatous tissue investing the bundle. From this it may be inferred that the dorsal space referred to as "phloem region" may have originally been fine parenchyma, as those surrounding the bundle, or some "secretory cells" claimed by SEWARD (7) to take the place of true phloem in *Lepidodendron*. Some of these spaces measure 0.25 mm. across, or occupy a larger area than the bundle. Whether this space represents a phloem, or not, we have the beginning of a collateral bundle here instead of in the cortex (4). From the way the sporophyll traces are arranged in the cortex the pedicels must have been spiral on the axis.

As represented in the diagram of a sporophyll (fig. 14), the pedicel rises with the axis at an oblique angle for a considerable distance before it swells up on the ventral side (fig. 10b). From this point up to a distance of 26 mm., the sporangium is attached on the adaxial surface of the pedicel, and inclines at about 20° angle with the axis. The pedicel at this region becomes winged and slightly keeled on the dorsal surface (fig. 10 c-g). Beginning with *d* there arise on the ventral side of both the expanding wings sclerenchymatous tissues, forming lateral cushions for the large sporangium (fig. 11 scl). A striking peculiarity of this structure is the bilobed condition as the more distal end of the attachment of the sporangium to the pedicel is reached (fig. 11 scl'). Fig. 11 at the right side shows the beginning of this bilobed condition.

Figs. 10 and 11 show that the pedicel sends out a distinct stalk to the sporangium, with a bounding layer continuous with the sporangium wall. Unfortunately none of the pedicel bundles at this region is cut transversely to show structure, but dark masses of the same size as those noticed lower in the pedicel indicate that there was no change in size or in form. Near and at the terminal ends of the wings are epidermal cells, but not as well defined as those figured by MASLEN. The "phloem space" could not be recognized in many of the sections, due perhaps to the crowded condition in this region.

As the periphery of the cone is reached, a broadened lamina rises upward from the widening distal end of the pedicel. This part tapers to a point high above its base. The actual length cannot be given, but, judging from the large and increasing number of lamina sections

as the apex is approached, and from the sporophyll reconstruction, it may measure 18 mm., thus justifying the specific name *foliaceus* given by MASLEN. A downward heel of the sporophyll lamina is represented in cross-section (fig. 1*h*) as triangular in shape. This is the only one identified as a downward heel; so it may be inferred that it is short.

The bundle at the base of the lamina (fig. 5) shows the same number of elements as previously noted. The elements here show scalariform markings, as they are cut slightly tangentially. A few fine parenchymatous cells surround the bundle, but outside these are scattered thick walled cells with scalariform thickenings. They are abundant on the ventral side, but absent on the dorsal side. These structures fit well the description of "transfusion tissue" or "short barred cells" (9). Toward the tip of the lamina the bundle becomes concentric, as the "phloem space" which accompanies it up to the region of the base of the lamina disappears as the apex is reached.

The striking feature of the species mentioned by MASLEN, the "indiscriminately scattered cells with dark contents" (or "secretory sacs"), is easily recognized. Fig. 8 shows the general distribution of these cells, while fig. 7 shows them in greater detail among the large celled parenchyma which makes up the greater part of the sporophyll lamina. Epidermal cells are recognized. Below these is a layer of hypoderma which is thicker along the middle of the lamina. Generally the ventral side has a thicker layer of hypoderma.

Fig. 12 shows the remains of a ligule between what apparently are extensions of the inner lobe of the lateral cushions beyond the sporangium stalk. Above the ligule is shown the sporangium wall wholly separated from the pedicel. This situation may be looked upon as though the ligule arises from a cavity under the projecting distal end of the sporangium, with the extensions of the inner lobe of the lateral cushions at the sides, and the ventral wall of the lamina on the outer side.

Sporangium

As already mentioned, the pedicel sends out a distinct stalk to the sporangium. This is continuous throughout the greater length of the pedicel, forming a longitudinal ridge of tissue between the sclerenchymatous cushions. This is sometimes known as the "sub-

archesporial pad." On top of this is laid a mass of sterile tissue. To this stalk is attached a large sporangium, the largest of which measures at least 27 mm. \times 2 mm.

The wall of the sporangium consists of the ordinary palisade-like cells, thicker on the dorsal side just above the wings of the pedicel than on the ventral side. At places where the wall bends sharply, a layer of cellular tissue is often observed. This can be traced to the sterile mass of tissue laid on top of the subarchesporial pad. Arising from the ridge formed by the sterile mass of the tissue is a delicate radial plate, recalling the trabeculae structures in the modern *Isoetes*. Fig. 10 shows that toward the proximal end such a structure is absent, but as section *e* is reached there appears such a structure bifurcating near the top. Section *g* or the more distal sections show distinctly these trabeculae. A survey of a section of the cone from the axis to the periphery reveals the same situation. The basal and apical sections of the cone show no variation from this plan. From this it looks as though ontogenetic development of this structure must have begun from the distal end, and that these are definite plates partly dividing the sporangium case into three regions.

Spores

The numerous spores (fig. 13) which fill the sporangium measure approximately 0.032 mm. in diameter. On close observation the tri-radiate markings are noticeable on those not broken in the preparation. A thorough search of all the sections failed to disclose a single megaspore. In a way this result sounds startling, for, as it will be recalled, Mrs. D. H. SCOTT (6) in 1906 designated *Triletes diabolicus* as belonging to *Lepidostrobus foliaceus* Maslen. BERRY (3), however, says that the two kinds of spores may be produced upon different cones. The specimen under investigation is as perfect as can be expected. A glance at the reconstruction (fig. 9) will suggest the idea that although the lowest slide does not represent the real base of the cone, the specimen very nearly represents the whole cone. The converging of the general outline toward the base, the total absence of sporophyll lamina sections on the first two lowest slides (G 18-19), and the decreased number of sporangia sections on these support this view. The writer feels sure, therefore, that the specimen on hand is homosporous.

Related Lepidostrobi

On the basis of structure, the closest relatives of *L. foliaceus* Maslen are *L. Oldhamius* Will. (1, 4, 8), of which there are many forms, *L. Veltheimianus* (5, vol. 1), *L. Bertrandi* (10), *L. Binneyanus* (1), and *L. gracilis* (1). These, as well as *L. foliaceus* Maslen, agree in all the generic characters of *Lepidostrobus* as given by ZEILLER (11).

Diagnosis

The following characters are enumerated for the diagnosis of *Lepidostrobus foliaceus* Maslen:

1. Lower Carboniferous Formation; Gannister Horizon.
2. The cone is ovoid, measuring 5.1×2.4 cm.
3. The sporophyll has a long triangular pedicel (26 mm.) up to the attachment of the sporangium, and leaves the axis at an oblique angle of about 20° .
4. A distinct stalk to the sporangium elongated along the length of the pedicel is recognized; hence the sporangium is not sessile.
5. The pedicel under the sporangium and on the wings has two lateral sclerenchymatous tissues, each of which is bilobed at the distal end, seemingly acting as supports to the massive sporangium.
6. The ligules arise from a cavity overhung by the distal end of the sporangium.
7. On top of the subarchesporial pad is a ridge of sterile tissue from which arise sterile plates or trabeculae.
8. The sporangia bear only microspores; hence the specimen is homosporous. Another form (6) is reported heterosporous.
9. The axis consists of a small pith, a rather broad zone of primary xylem with peripheral protoxylem points, a narrow meristematic region, and an undifferentiated cortex imbedding sporophyll trace bundles.
10. The bundles consist of 30-70 scalariform elements, accompanied on the dorsal side with a "phloem space" beginning at the base of the pedicel as it leaves the axis, up to the region of the base of the sporophyll lamina where transfusion tissues appear.
11. The lamina of the sporophyll is composed mostly of large celled parenchyma, many cells of which show dark contents simulating "secretory sacs."

12. The upturned lamina is unusually long, attaining a length of 18 mm. in some cases.

From this diagnosis it is clear that the material studied is unmistakably identical with that partly diagnosed by MASLEN. It will be fair, therefore, to call it *L. foliaceus* Maslen. Fortunately the specimen is in excellent preservation to supply the needed structures in fine details, so that a full account of the species is now available.

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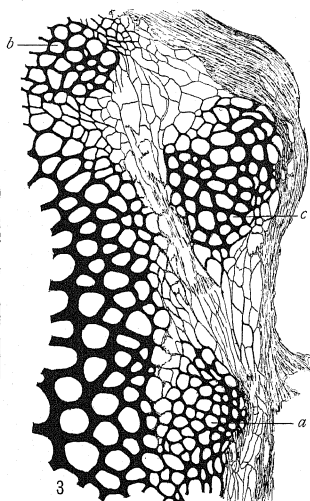
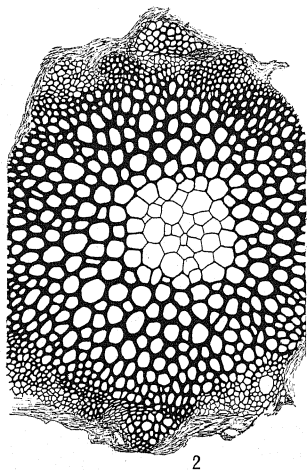
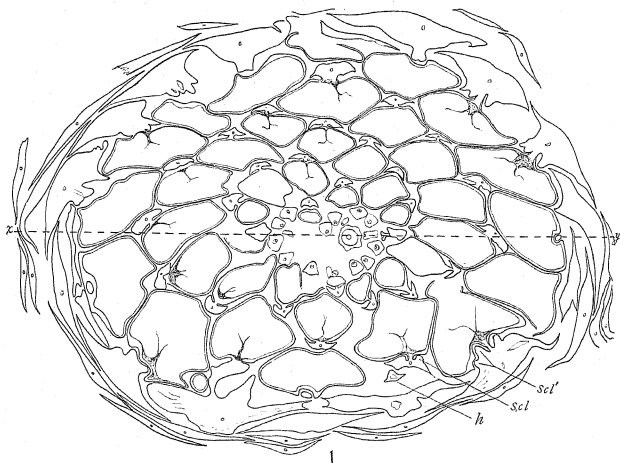
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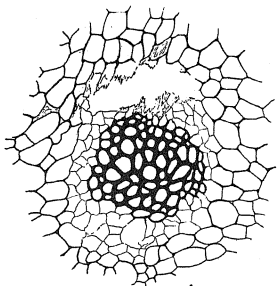
EXPLANATION OF PLATES XXX-XXXII

FIG. 1.—Transverse section through middle of cone (G 14), showing axis in middle surrounded with 17 pedicels, 2-3 rows of sporangia, and number of sporophyll lamina sections outside: *h*, heel of sporophyll lamina; *scl*, sclerenchymatous cushion; *scl²*, bilobed sclerenchymatous cushion; $\times 4$.

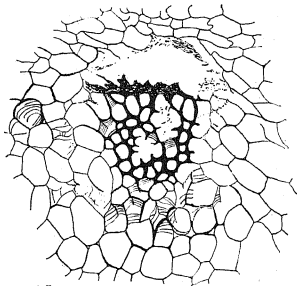
FIG. 2.—Transverse section of cone axis near base (G 18), showing narrow pith in center, rather broad primary xylem cylinder bordered with protoxylem



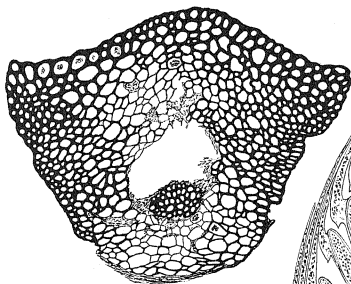




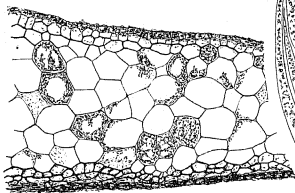
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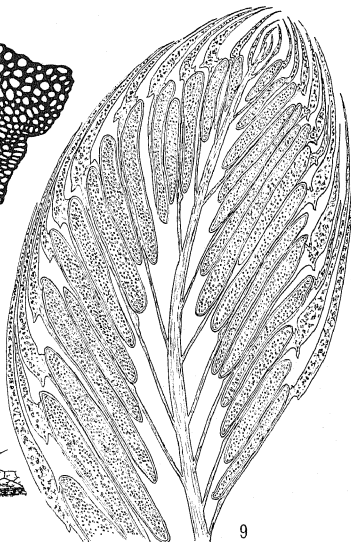
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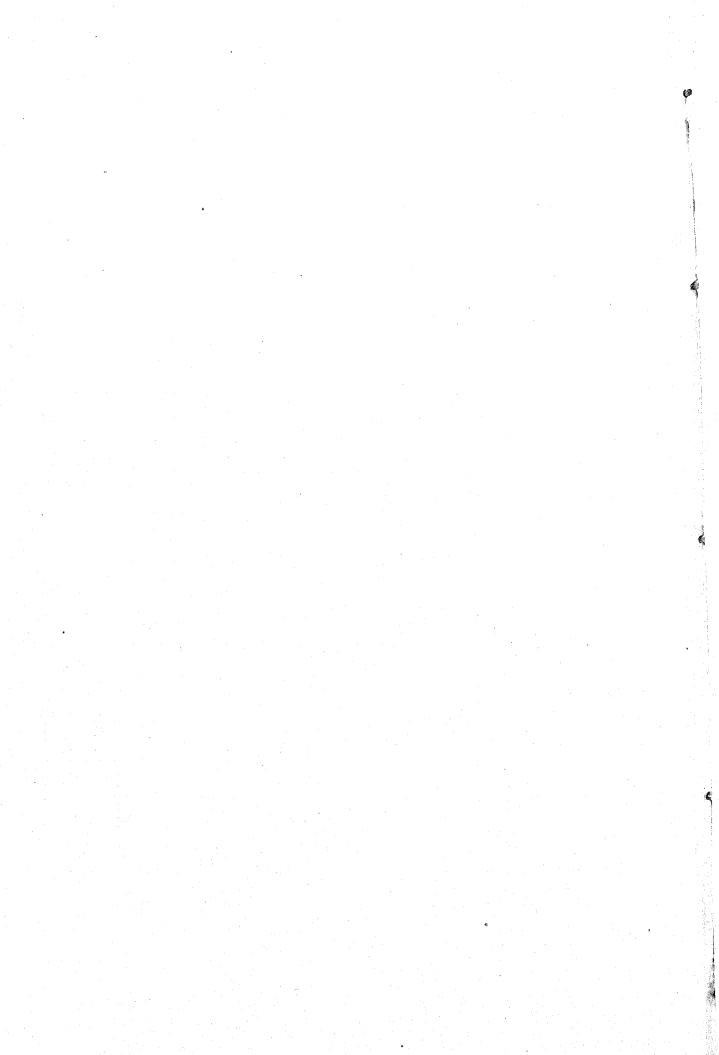
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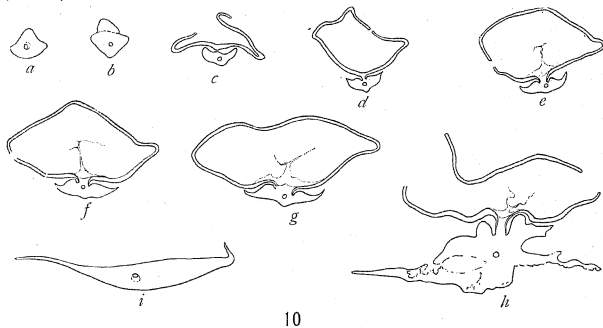


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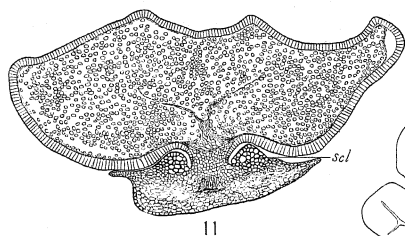


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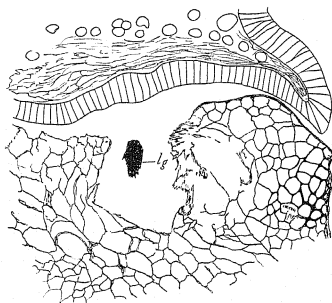
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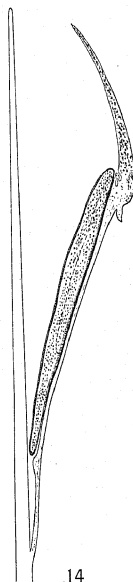
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points, 1-2-celled meristematic region, and narrow undifferentiated cortex containing sporophyll traces; $\times 73$.

FIG. 3.—Details through outer portion of axis (G 18 at B), showing portion of xylem cylinder: *a*, protoxylem point just beginning to separate; *b*, bundle being cut off by insertion of meristematic cells; *c*, bundle wholly separated from xylem cylinder but still imbedded in cortex; $\times 196$.

FIG. 4.—Details of pedicel bundle (G 11), showing on dorsal side (above) a phloem region, and thin walled parenchyma surrounding simple bundle; $\times 196$.

FIG. 5.—Details of bundle (G 9 at E) at base of lamina, showing transfusion tissue on ventral side (below); $\times 196$.

FIG. 6.—Transverse section of pedicel (G 18 at C) below sporangium attachment; "phloem space" above bundle; $\times 73$.

FIG. 7.—Details of transverse section of sporophyll lamina (G 11 at C); epidermis partly disorganized but hypoderma well preserved; among large celled parenchyma are indiscriminately scattered cells with dark contents or "secretory sacs"; $\times 73$.

FIG. 8.—Transverse section of sporophyll lamina (G 11 at C), showing general distribution of "secretory sacs"; $\times 21$.

FIG. 9.—Reconstruction of median longitudinal section of whole cone based upon twelve transverse sections (G 8-19); $\times 2$.

FIG. 10.—Series of sections of single sporophyll with sporangium (G 11-19): *a*, from G 19; *i*, from G 11; $\times 8$.

FIG. 11.—Details of transverse section of pedicel and attached sporangium (G 9 at D); *sc*, sclerenchymatous cushion; $\times 21$.

FIG. 12.—Transverse section of distal portion of sporangium and pedicel (G 10 at D), showing ligule (*lg*) between extensions of bilobed sclerenchymatous cushions; $\times 73$.

FIG. 13.—Microspores showing characteristic triradial markings; *a*, from G 8 or apex of cone; *b*, from G 14 or middle; *c*, from G 19 or near base of cone; $\times 363$.

FIG. 14.—Reconstruction of sporophyll bearing sporangia adaxially (based upon series of sections sketched in fig. 10); sporophyll as shown inclines at 20° angle with axis; $\times 2$.

BRIEFER ARTICLES

PARADICHLOROBENZENE IN THE HERBARIUM

The compound paradichlorobenzene, for which GUYTON and STEAR¹ have recently proposed the convenient and sensible abbreviation PDB, has lately come into extensive agricultural use as an agent for killing peach borers. It comes on the market in the form of volatile, whitish crystals giving off at ordinary temperatures a gas with a penetrating and pungent but not unpleasant odor, which is highly poisonous to insects. In this laboratory it has been tested for a year and a half in the mycological herbarium, and has been found to possess certain distinct advantages over other material used to protect fungi from insect attack. It has been found particularly valuable for disinfecting large boxes of specimens awaiting detailed study, and the large packages of material kept for class use, both of which are particularly likely to be attacked by insects because they are necessarily stored in such a way as to afford less protection than is given to regular herbarium specimens.

The gas is heavier than air; hence in disinfecting large boxes of material known or suspected to be infested with insects, the specimens are first placed in the box, then a small quantity of the PDB crystals is put on top in a folded paper or an envelope, and the box closed. When the space to be disinfected is small, the position of the crystals is unimportant. The specimens need not be removed from their envelopes unless time is pressing, in which case, if the specimens are unwrapped and a somewhat larger quantity of the crystals used, results will be secured more rapidly.

PDB is much more volatile than naphthalene, and will not entirely replace the latter for permanent use in the packets. In cold weather it has been found desirable to use the two together. A few crystals of PDB are added to the usual quantity of naphthalene placed in each packet; or, if a large number of specimens is being put up at one time, the two are mixed together in the proportion of one part of PDB to two or three of naphthalene, and a pinch of the mixture is put into each packet. It is not desirable to mix the two in hot weather, since the mixture melts at 40° C. and begins to cake at an even lower temperature, while the PDB alone remains solid up to 50° C. The compound is quite harmless to the specimens, and in the small quantities used seems to have no effect whatever upon human beings. It has been found so convenient and effective in the limited field in which it has been tried as to suggest that its use might well be extended to the protection of preserved specimens of other groups of plants.—G. W. MARTIN, *University of Iowa, Iowa City, Iowa.*

¹ Penna. Dept. Agric. 1924. General Bull. 383.

CURRENT LITERATURE

BOOK REVIEWS

Colloid symposium monograph

Attention of plant physiologists is called to the publication of the *Second colloid symposium monograph*,¹ which contains the papers presented at Northwestern University during the summer of 1924. There are twenty titles, some of which are worthy of note. Among these may be mentioned a paper by MICHAELIS on *The general principles of the effects of ions in colloids*, and one by FREUNDLICH on *Sols with non-spherical particles*. Several papers deal with emulsification. These are by HOLMES and WILLIAMS on *Polar emulsifying agents*, and Iodine as an emulsifying agent; and a paper by HARKINS on *The orientation of molecules in the surfaces of liquids*.

The outstanding contribution is a lengthy discussion of the prolamines and their chemical composition in relation to acid and alkali binding, by HOFFMANN and GORTNER. This excellent paper occupies about 150 pages, and constitutes what HOLMES calls "a masterly reply to certain implied criticisms of colloid chemistry found in LOEB's book on proteins and the theory of colloidal behavior."

Two types of combination between proteins, and acid and alkali, are recognized. Chemical combination holds between hydrogen ion concentration limits from P_H 2.5 to P_H 10.5, and between these limits the amount of acid or alkali bound depends on the chemical composition of the protein. When the acidity is greater than P_H 2.5, or alkalinity greater than P_H 10.5, however, an adsorption type of combination occurs; and at these extreme ranges all types of prolamines, regardless of their composition, bind approximately the same amount of acid or alkali.

Indications are found that the isoelectric "point" of a protein is really an isoelectric range, the position of the range on the P_H scale depending on the chemical composition of the protein. The *calculated* isoelectric points obtained by extrapolating the logarithmic curves for acid and alkali binding, however, are found to fall near the neutral point of water, P_H 7, which would be expected on the theory that at higher concentrations of acid and alkali, the combination follows the adsorption law. These calculated isoelectric points, therefore, are not related to the chemical composition of the protein. This paper deserves careful study by all who desire to keep abreast of our knowledge of the physical chemistry of the proteins.—C. A. SHULL.

¹ Colloid symposium monograph. Vol. II. Ed. by H. N. HOLMES. 8vo. pp. viii + 368. N.Y.: Chemical Catalog Co.

MINOR NOTICES

Tree habits.—For the student of forest life who wants something more than a diagnosis of the species, a recent book by ILLICK² on tree habits will be welcomed. Those who desire to increase their ability to recognize the hardwoods at all seasons of the year may learn much from its pages. The writer seems to have attained a happy medium of style that lies between the dryness of mere taxonomic description and the sentimentality that characterizes some so-called "nature books." The book reads easily, while the information is scientifically accurate. The identification of the species is aided by illustrations, keys, and tables. In the latter are included many details of habit and structure that are seldom mentioned in textbooks, but are in continual use by ecologists, foresters, and lumbermen. The illustrations are numerous and excellent, including drawings of leaves, flowers, and fruits, and photographs of trunks, twigs, and entire trees. The glossary, keys, and index are adequate, although the last should include scientific as well as common names. While the book does not include all our broad-leaved trees, few species of wide distribution or of any economic importance are omitted. It is hard to conceive of a volume of this type that is likely to prove more companionable.—GEO. D. FULLER.

NOTES FOR STUDENTS

Temperature for ripening of sweet corn.—Not all are in agreement as to what the chemical composition of sweet corn should be at the time it is picked to be eaten, or to be packed into cans. Usually the sweetness is emphasized, but some state that the content of starch and crude fiber is of equal if not of greater importance. APPLEMAN and EATON³ have determined the chemical changes in sweet corn during ripening, relating these to temperature, and attempting to make the results of some practical value as a guide for the picking of sweet corn in different parts of the country, or in different seasons in the same locality. The decrease in starch and the increase in sugar were the chief changes noted. At the time of greatest sugar content, the reducing sugars predominate, but these quickly decrease in amount. Since the reducing sugars are not nearly as sweet as cane sugar, the stage of greatest sweetness is not that of greatest total sugar content, but that of greatest cane sugar content. The changes in the amount of fat, crude fiber, and total nitrogen occurred early in the ripening process, and after that there was not much change in the amount of these constituents present. The rate of ripening is controlled by temperature. It took fifteen days for a late crop to reach the same stage of ripening that was reached by an early crop in six days. The temperatures of the two periods were compared, and after trying out several temperature indices, in an attempt to evalu-

² ILLICK, J. S., *Tree habits: how to know the hardwoods*. pp. iv+337. figs. 196. Amer. Nature Assoc., Washington, D.C. 1924. \$4.00.

³ APPLEMAN, C. O., and EATON, S. V., *Evaluation of climatic temperature efficiency for the ripening processes in sweet corn*. Jour. Agric. Res. 20: 795-805. 1921.

ate the efficiency of temperature as the controlling factor in the ripening process, it was decided that the exponential indices furnished the best criterion. The time ratio for the ripening periods of the two crops was practically the same as the ratio of the averages of the daily exponential indices for the two periods. This ratio was 2.57, so the rate of ripening seems to follow the Van't Hoff-Arrhenius law. The exponential indices were used to predict for different localities and different seasons of the same locality the maximum number of days that sweet corn will remain in the best edible condition, as well as the approximate number of days required for corn to pass from the beginning of kernel formation to the stage of best quality for canning or edible purposes. These results should be of practical value to cannery of sweet corn.—S. V. EATON.

The Sargasso Sea.—A recent report by WINGE⁴ seems to add materially to our knowledge of this curious plant community. The vegetation is represented as varying much in abundance, from miles of open sea without a plant, through thin layers of drift, to rather dense masses of tangled plants. Doubt is expressed whether any of the drifting *Sargassum* belongs to anchored shore species. Eight floating forms or species are described and designated by the numbers I to VIII, three of these forming the great mass of the vegetation. It is suggested that these be transplanted to the shallow waters of the coast in order to determine their relation to coastal forms. *Sargassum* requires a water temperature of 18° C. in order to thrive, and this must be considered in experimental work.

The extent of the Sargasso Sea has been mapped, and it has been determined that its northern boundary varies seasonally, reaching farther north during the summer, dependent on the direction of prevailing winds. The other boundaries are relatively fixed.—GEO. D. FULLER.

Floral ontogeny.—THOMPSON⁵ has contributed the first paper in a new series of botanical publications to be issued by the University of Liverpool. He states that "in the study of flowering plants attention has been so focussed on the adult organism that the facts of ontogeny have for the most part been passed unnoticed." In this initial study the floral ontogeny of the Amherstieae (Leguminosae) is described in great detail and well illustrated. The general purpose is to discover in this way suggestions as to relationships. As a result of this study of the tribe Amherstieae, the genera are rearranged according to the records of their ontogenies.

Floral ontogeny was first established by PAYER in 1857, but has been very

⁴ WINGE, Ö., The Sargasso Sea, its boundaries and vegetation. Rept. Danish Oceanographical Exp. 1908-10 to the Mediterranean and adjacent seas. 3^r: pp. 34. figs. 14. 2 maps. 1923.

⁵ THOMPSON, J. M., Studies in advancing sterility. I. The Amherstieae. Univ. Liverpool, Publ. Hartley Bot. Lab. no. 1. pp. 54. figs. 104. 1924.

little developed since, although it is a subject well deserving cultivation. It is significant, therefore, that this field of research has been opened again in this series of studies to be published by the University of Liverpool.—J. M. C.

Trees and shrubs of Mexico.—STANDLEY⁶ has published the fourth installment of the *Trees and shrubs of Mexico*, bringing together in organized form the known material of 37 families. This work has involved the study of 275 genera and 1738 species. Very few new genera and species are included, as the work is intended to assemble rather than to monograph the groups. One new genus (*Leucopremna*) of Caricaceae is established, and 43 new species, 11 of which belong to *Eugenia* (Myrtaceae). Much the largest family is the Cactaceae, since it is displayed more in Mexico than in any other region of the world. This family is presented by BRITTON and ROSE, and includes 54 genera and 523 species. The largest genera are *Neomammillaria* with 134 species, *Opuntia* with 87, and *Echinocereus* with 48. The other large families are Menthaceae (127 species), Asclepiadaceae (119 species), Solanaceae (114 species), and Melastomaceae (114 species).—J. M. C.

Single fertilization in barley.—HARLAN and POPE⁷ have discovered some strains of barley in which certain abnormalities appeared in seed development, notably a few seeds lacking an embryo, and a much greater number deficient in endosperm. These seeds were investigated, and the conclusion reached that they were to be explained by failure of "double fertilization." No cytological investigation was undertaken, which might discover this variable behavior of the two "male cells."—J. M. C.

Embryo of *Linum*.—SOUÈGES⁸ has published the results of his investigation of the embryogeny of *Linum catharticum*, with his usual fullness of detail and clear illustrations.—J. M. C.

⁶ STANDLEY, P. C., *Trees and shrubs of Mexico* (Passifloraceae-Scrophulariaceae). Contrib. U.S. Nat. Herb. 23:840-1312. 1924.

⁷ HARLAN, H. V., and POPE, M. N., Some cases of apparent single fertilization in barley. Amer. Jour. Bot. 12:50-53. 1925.

⁸ SOUÈGES, R., Développement de l'embryon chez le *Linum catharticum* L. Bull. Soc. Bot. France IV. 24:925-938. 1924.

GENERAL INDEX

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